This Page Is Inserted by IFW Operations and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of The original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents will not correct images, please do not report the images to the Image Problem Mailbox.

WORLS INTELLECTUAL PROPERTY ORGANIZATION International Bureau

Document **AM4** Appl. No. 09/677,574

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: (11) International Publication Number: WO 95/14782 C12N 15/54, 9/12, 15/10, 1/21, C12Q 1/68 A2 (43) International Publication Date: 1 June 1995 (01.06.95) (21) International Application Number: PCT/US94/13554 (81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH. CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IP, KE, KG, (22) International Filing Date: KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TI, TT, 22 November 1994 (22.11.94) UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, (30) Priority Data: ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI 08/156,020 23 November 1993 (23.11.93) patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, US SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

08/156,020 (CIP)

23 November 1993 (23,11,93)

Published

(60) Parent Application or Grant (63) Related by Continuation

US Filed on

(71) Applicant (for all designated States except US): STATE OF OREGON acting by and through THE OREGON STATE BOARD OF HIGHER EDUCATION on behalf of THE OREGON HEALTH SCIENCES UNIVERSITY [US/US];

3181 S.W. Sam Jackson Park Road, Portland, OR 97201-3098 (US). (72) Inventor; and

(75) Inventor/Applicant (for US only): MOSES, Robb, Edwin [US/US]; 2771 S.W. Patton Lane, Portland, OR 97201 (US).
 (74) Agent: GREENFIELD, Michael, S., Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).

(54) Title: A MODIFIED THERMO RESISTANT DNA POLYMERASE

(57) Abstract

Novel, modified Taq DNA polymerases and genes encoding for them are disclosed. The modified Taq DNA polymerases of the invention are the same size, have the same heat stability and synthesis rate as the native enzyme, but lack the 5'-3' exonuclease activity. As a result of this modification, the enzymes have improved processivity as compared to the native enzyme. The enzymes of the present invention enable improved methods of conducting PCR, DNA sequencing, and DNA synthesis.

Eco RI Kpnl BstXI

PUC18

Mutagenesis Trestments 1-4

PCR

R₁ Kpnl R₁ BstXI

CREM-T2)

CREM-T2)

CREM-T2)

Identification of REM-T3

Without international search report and to be republished

upon receipt of that report.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

			44.1.4.4.4	140	Administration 1
ΑT	Austria	GB	United Kingdom	MR	Macricania
AU	Agstralia	GΕ	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	Œ	Ireland	NZ	New Zealand
B.J	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic	ŞD	Sudan
CG	Congo		of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	Sī	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
cs	Czechoslovakia	LU	Luxembourg	TG	Togo
cz	Czech Republic	LV	Lervia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldovs	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FD	France	MN	Mongolia	VN	Viet Num

WO 95/14782 PCT/US94/13554

1

MODIFIED THERMO-RESISTANT DNA POLYMERASES

BACKGROUND OF THE INVENTION

"This invention was made with Government support under grant GM 24711 awarded by the National Institutes of Health. The Government has certain rights in the invention."

5 Field of the Invention

10

15

20

-J.

This invention relates to the field of DNA polymerases for use in the polymerase chain reaction and DNA sequencing.

Description of the Prior Art

Polymerase Chain Reaction (PCR) was one of the most important inventions developed in area of biotechnology during the 1980's and has proven useful for a variety of tasks. PCR Technology, Principles and Applications for DNA Amplification (Erlich ed. 1989). The process provides a method for amplifying known specific nucleic acid sequence. Mullis, U.S. Pat. No. 4,683,202. The process comprises treating single or double stranded DNA containing the sequence of interest with an excess of two oligonucleotide primers sufficiently complementary of the strands so as to hybridize to the denatured strands. The hybridized primers are then extended by a DNA polymerase in the presence of the four dNTPs. The primer extension products are then separated and can serve as templates for another cycle of replication. The number of DNA templates approximately doubles on each cycle of amplification. Thus, 20 cycles of the process will result in approximately a 220-fold amplification.

The original protocols for PCR used the Klenow fragment of E. coli DNA polymerase I to catalyze the extension of the oligonucleotide primers. Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51, 263 (1986); Mullis and Faloona, Methods

10

15

20

٠,

Enzymol. 155, 335 (1987). The Klenow fragment proved somewhat cumbersome to use. Denaturation of the double stranded DNA at the start of each cycle requires temperatures ranging from 80 to 105°C. These temperatures inactivate the Klenow fragment. Consequently, fresh enzyme was required at the start of each new amplification cycle. While this process generally worked well for small segments of DNA (< 200 bp), a host of problems arose when replication of larger fragments was attempted.

The difficulties associated with use of the Klenow fragment DNA polymerase were circumvented with the introduction of thermostable DNA polymerase obtained from the thermophilic bacterium *Thermus aquaticus* (*Taq* DNA polymerase). Saiki et al., *Science* 239, 487 (1989); Gelfand et al., U.S. Pat. No. 4,889,818. This enzyme has been cloned, overproduced, and the DNA sequence determined. Lawyer et al., *J. Biol. Chem.* 264, 6427-6437 (1989).

In addition to its DNA polymerase activity, *Taq* DNA polymerase also possesses 5 '-3' polymerization-dependent exonuclease activity, but it lacks 3 '-5' exonuclease activity. Longley et al., *Nuc. Acids Res.* 18, 7317-7322 (1990); Blanco et al., *Gene* 100, 27-38 (1991); Bernad et al., *Cell* 59, 219-228 (1989); Lawyer et al., *supra*; Holland et al., *Proc. Natl Acad. Sci.* 88, 7276-7280 (1991); and Kelly and Joyce, *J. Mol. Biol.* 164, 529-560 (1983). Studies have identified the 5 '-3' exonuclease activity as being an intrinsic part of *Taq* DNA polymerase. Longely et al., *supra*; and Barnes et al., *Gene* 112, 29-35 (1992). This activity appears to facilitate a nick translation DNA reaction.

Native Taq DNA polymerase suffers from a high rate of misincorporation — about four times higher than that of the Klenow fragment of E. coli DNA polymerase I.

÷

-:

AmpliTaq™ is a

It has been estimated that *Taq* DNA polymerase incorporates one incorrect nucleotide in 9000. Tindall and Kunkel, *Biochemistry* 27, 6008 (1988). After 20 amplification cycles, this would result in DNA molecules with random mutations averaging one in every 900 bases. Saiki et al., *supra*. If the PCR product is to be inserted into an expression vector, the chance that one cloned molecule will contain an unwanted sequence alteration may be significant. It would be desirable, therefore, to decrease the rate of misincorporation of the DNA polymerase used in PCR without sacrificing the heat stability and rate of synthesis of the native *Taq* DNA polymerase.

10

15

5

It has been shown that removal of the 5'-235 codons of the *Taq* DNA polymerase gene results in an expression product that has no 5'-3' exonuclease activity and a lower rate of mutagenesis. Tindall et al., *supra*; and Barnes, *supra*.

commercially available genetically engineered version of *Taq* DNA polymerase and is substantially equivalent to the native form. Perkin Elmer Cetus; Saiki and Gelfand, *Amplifications* (Perkin Elmer Cetus), 1, 4 (1989). Also commercially available is a truncated gene product, the Stoffel fragment, that expresses an enzyme lacking the 5 '-3' exonuclease activity and having much lower unit activity, probably due to decreased processivity and increased mutagenesis. Barnes, *supra*. Gelfand

Other forms of Taq DNA polymerase are available.

20

and Abramson (PCT International Publication No. WO 92/06200) disclosed a modified *Taq* polymerase having the same length as the native enzyme, but with highly attenuated 5 '-3' exonuclease activity. The exonuclease activity is defeated by mutation in nucleotide 137 of the *Taq* polymerase gene, wherein the mutation is G

to A, resulting in a change in amino acid 46 of the enzyme from Gly to Asp. This enzyme is reported as having the same polymerase activity, processivity and extension rate as the native enzyme.

SUMMARY OF THE INVENTION

An object of this invention is to enhance the synthesis activity of DNA polymerase as used in PCR and DNA sequencing.

5

10

15

20

The invention disclosed herein achieves this object by providing a modified *Taq* DNA polymerase and a correspondingly modified *Taq* DNA polymerase gene sequence. The modified *Taq* DNA polymerase is the same size, has the same heat stability and synthesis rate as the native enzyme, but the 5 '-3' exonuclease activity is missing. As a result of this modification, the gene expression product has improved processivity.

The enzymes of the present invention enable improved methods of conducting PCR, DNA sequencing and DNA synthesis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical depiction of the restriction map of the Taq DNA polymerase gene.

Figure 2 is a graphical depiction of the method for producing the modified Taq DNA polymerase and the gene encoding it.

Figure 3 shows the sequencing primers for the pLSM5 (SEQ ID NO: 3) plasmid.

Figure 4 is a schematic depiction of the method for testing processivity used in trials 1 and 2.

Figure 5 is the autoradiograph showing the results of processivity testing used in trial 1.

Figure 6 is the autoradiograph showing the results of processivity testing used in trial 2.

5

15

20

Figure 7 is a schematic depiction of the method for testing processivity using PCR.

Figure 8 is the autoradiograph showing the results of processivity testing by the PCR method.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "replication product" refers to the oligonucleotides synthesized by DNA polymerase, whether it be as part of the polymerase chain reaction, DNA sequencing, or any other reaction where DNA polymerase is used to synthesize an oligonucleotide.

The term "oligonucleotide" as used herein is defined as a molecule composed of two or more deoxyribonucleotides or ribonucleotides.

The term "thermostable" refers to an enzyme that is stable to heat (> 95°C) and catalyzes combination of nucleotides to form an oligonucleotide. The term "thermo stability" as used herein refers to the characteristic stability of an enzyme to heat.

As used herein, the term "altered amino acid" means an amino acid that differs from that found in the native peptide or protein. Hence, if the native peptide

10

15

20

has the amino acid Cys at position 43, and the modified peptide has the amino acid Gly at that position, Gly is the "altered amino acid." Similarly, the term "altered nucleotide" means a nucleotide that differs from that found in a native oligonucleotide, polynucleotide, gene, or other nucleotide fragment.

As used herein, the phrase "lacking 5'-3' exonuclease activity" means an enzyme having less than 1% of the 5'-3' exonuclease activity of the native *Taq* DNA polymerase.

We undertook to inactivate the 5 '-3' exonuclease activity of the *Taq* DNA polymerase by *in vitro* mutagenesis without removal of the portion of the gene encoding that activity. The procedure followed was to develop a method of "zone mutagenesis" for that region of the *Taq* DNA polymerase gene encoding for the 5'-3' exonuclease activity. *See* Figure 2. The nucleic acids encoding the amino acid residues required for 5'-3' exonuclease activity have not been clearly identified, but earlier work suggests the region involved in DNA polymerases from other bacteria. Kelly and Joyce, *supra*.

To briefly summarize, using PCR technology we generated a *Taq* gene, which we cloned into the plasmid vector pUC18. *See* Figure 1. The pUC18 plasmid containing the *Taq* gene is designated pLSM5 (SEQ ID NO: 3). There are four base changes in the *Taq* gene produced by PCR and cloned in pLSM5 (SEQ ID NO: 3) compared to the published *Taq* DNA polymerase gene sequence (TTHTAQPIA in GenBank) (SEQ ID NO: 1): 1) C to G at position 89 in the untranslated 5 end, 2) T to A at position 934 (Phe to Ile), 3) T to C at position 962 (Leu to Pro), and 4) G to A (no amino acid change) at position 2536. The protein expression product of this

20

25

gene has an altered amino acid at positions 272 (Ile) and 281 (Pro). We then subjected the pLSM5 (SEQ ID NO: 3) plasmid to conditions that would cause the random mutations in the 5° exonuclease domain.

The vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) begins at nucleotide 70 and ends at 2619. The reading frame for translation begins at nucleotide 121 and ends at 2619 by the convention of Lawyer et al., *J. Biol. Chem.* 264, 6427-6437 (1989).

The following sequence appears at the 5 junction between the pUC18 plasmid and the Taq gene:

10 ... AATTTCACACAGGAAACAGCTATGACCATGATTACG<u>AA</u>TTCTAAA ... (SEQ ID NO: 14)

This sequence begins with the pUC18 antisense nucleotide sequence 490 to 455. The underlined nucleotides (AA) were added to create a restriction site. The *Taq* gene sequence (bold face) begins at nucleotide 70).

The following sequence appears at the 3° junction between the pUC18 plasmid and the *Taq* gene:

....CAAGGAGTGAGATTCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTT ... (SEQ ID NO: 15)

This sequence begins with *Taq* polymerase gene nucleotide 2610 to 2619. The underlined nucleotides (GA) were added to create a restriction site. The remaining sequence is the pUC18 antisense nucleotide, 413 to 381. Both junction sequences have been verified by sequence analysis.

The enzyme expression product of the pLSM5 plasmid, REM-T2 (SEQ ID NO: 4), has substantially the same processivity, 5:-3 exonuclease activity, and

10

15

20

performance in normal PCR, to the extent tested so far, as the commercially available *Taq* DNA polymerase AmpliTaq™.

A variety of methods of mutagenesis are known to those of skill in the art and may be used in preparing a modified Taq DNA polymerase gene according to the present invention. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 2d Ed. 1989). The mutated genes were then treated with restriction endonucleases that cut it in the region believed to be responsible for 5 '-3' exonuclease activity, thereby producing mutated inserts coding for that portion of the gene. A vector containing the native Taq DNA polymerase gene was treated with the same endonucleases and the previously obtained inserts ligated into the vector. Cells were transformed with the vector containing the inserts and colonies grown. We assayed polymerases expressed by the various colonies for polymerase activity as well as 5 '-3' exonuclease activity. The cells transfected with the gene encoding the modified Taq DNA polymerase meeting the objective of the present invention were thereby identified.

Appropriate host cells for the present invention may be chosen from the prokaryote group, which most frequently are represented by various strains of *E. coli*. Other microbial strains such as bacilli may be used, however. *Bacillus subtilis* and various species of *Pseudomonas* may be used, for example.— In such prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species by Bolivar, et al., *Gene* 2, 95 (1977). pBR322 contains genes for ampicillin and tetra-

10

15

20

÷

ş

cycline resistance, and thus provides addition markers that can be either retained or destroyed in constructing the desired vector. Commonly used prokaryotic control sequences, which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequence, include such commonly used promoters as the β -lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., Nature 198, 1056 (1977)), the tryptophan (trp) promoter system (Goeddel, et al., Nucleic Acids Res. 8, 4057 (1980)), the lambdaderived P_L promoter (Shimatake et al., Nature 292, 129 (1981)), and the N-gene ribosome binding site, which has been made useful as a portable control cassette (U.S. Pat. No. 4,711,845). The N-gene ribosome binding site comprises a first DNA sequence that is the P_L promoter operably linked to a second DNA sequence corresponding to N_{RBS} upstream of a third DNA sequence having at least one restriction site that permits cleavage within six bp 3 $\dot{}$ of the N_{RBS} sequence. Also useful is the phosphatase A (phoA) system described by Chang et al. in European Patent Publication No. 196,864 published Oct. 8, 1986. Any available promoter system compatible with prokaryotes can be used, however.

In addition to bacteria, eucaryotic microbes, such as yeast, may also be used as hosts. Laboratory strains of Saccharomyces cerevisiae, Baker's yeast, are most used, although a number of other strains are commonly available. While vectors employing the 2 micron origin of replication are illustrated (Brach, Meth. Enz. 101, 307 (1983)), other plasmid vectors suitable for yeast expression are known (see, e.g., Stinchcomb et al., Nature 282, 39 (1979), Tschempe et al., Gene 10, 157 (1980), and Clarke et al., Meth. Enz. 101, 300 (1983). Control sequences for yeast vectors include promoters

WO 95/14782 PCT/US94/13554

-.

5

10

15

20

10

for the synthesis of glycolytic enzymes. Hess et al., I. Adv. Enzyme Reg. 7, 149 (1968) and Holland et al., Biotechnology 17, 4900 (1978).

Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255, 2073 (1980) and those for other
glycolytic enzymes, such as glyceraldehyde-3-phosphate dehydrogenase, hexokinase,
pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose
isomerase, and glucokinase. Other promoters that have the additional advantage of
transcription controlled by growth conditions are the promoter regions for alcohol
dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated
with nitrogen metabolism, and enzymes responsible for maltose and galactose
utilization. Holland, supra.

It is also believed that terminator sequences are desirable at the 3° end of the coding sequences. Such terminators are found in the 3° untranslated region following the coding sequences in yeast-derived genes. Many of the vectors illustrated contain control sequences derived from the enolase gene containing plasmid peno46 (Holland et al., *J. Biol. Chem.* 256, 1385 (1981) or the LEU2 gene obtained from YEp13 (Broach et al., *Gene* 8, 121 (1978). Any vector containing a yeast-compatible promoter, origin of replication, and other control sequence is suitable, however.

It is also possible to express genes encoding polypeptides in eucaryotic host cell cultures derived from multicellular organisms. See, e.g., Tissue Culture (Cruz and Patterson eds., Academic Press 1973). Useful host cell lines include murine myelomas N51, VERO and HeLa cells, and Chinese Hamster Ovary (CHO) cells.

10

15

20

Expression vectors for such cells ordinarily include promoters and control sequences compatible with mammalian cells such as the commonly used early and late promoters from Simian Virus 40 (SV 40) (Fiers et al., *Nature* 273, 113 (1978)) or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses, or immunoglobulin promoters and heat shock promoters. A system for expressing DNA in mammalian systems using the BPV as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. General aspects of mammalian cell host system transformations have been described by Axel, U.S. Pat. No. 4,399,216. It now appears that "enhancer" regions are important in optimizing expression. These generally are sequences found upstream of the promoter region. Origins of replication may be obtained from viral sources. Integration into the chromosome, however, is a common mechanism for DNA replication in eucaryotes.

Plant cells are also now available as hosts. Control sequences compatible with plant cells such as the nopaline synthase promoter and polyadenylation signal sequence are available. Depicker et al., J. Mol. Appl. Gen. 1, 561 (1982).

In addition, expression systems employing insect cells utilizing the control systems provided by baculovirus vectors have been described. Miller et al, Genetic Engineering 8, 277-297 (Setlow et al. eds. Plenum Publishing 1986). These systems are also successful in producing Taq DNA polymerase.

Cells transformed with the modified *Taq* DNA polymerase gene may be grown using any suitable technique. The appropriate technique will depend on the cell type and will be known to those skilled in the art.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The treatment employing calcium chloride is used for prokaryotes or other cells that contain substantial cell wall barriers. Cohen, Proc. Natl. Acad. Sci. (USA) 69, 2110 (1972). Infection with Agrobacterium tumefaciens is used for certain plant cells. Shaw et al. Gene 23, 315 (1983). For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb is preferred. Virology 52, 546 (1978). Transformations into yeast are carried out according to the method of Van solingen et al., J. Bact. 130, 946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA) 76, 3829 (1979).

10

5

Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques that are well understood in the art. Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and relegated in the form desired.

15

20

Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions that are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. See, e.g., New England Biolabs, Product Catalog. In general, about 1 μ g of plasmid or DNA sequence is cleaved by one unit of enzyme in about 20 μ l of buffer solution. Often excess of restriction enzyme is used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C are workable, although variations are tolerable. After each incubation, protein is removed by extraction with phenol/chloroform, and may be followed by ether extraction. The nucleic acid may be recovered from

aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in *Methods in Enzymology* 65, 499-560 (1980).

5

Cells producing enzyme of the desired type can be identified by standard techniques for assaying DNA polymerase and 5 '-3' exonuclease activity. *Id.* Using some of these methods, we were able to isolate a *Taq* DNA polymerase having the same size, heat stability, and synthetic activity of native *Taq* DNA polymerase, but having increased processivity and resulting in decreased mutagenesis of PCR DNA products. *See* examples *infra*.

10

The modified *Taq* DNA polymerase of the present invention was chosen from a colony producing the enzyme with a relatively high polymerase activity and low 5 - 3 exonuclease activity. We designated this product REM-T3 (SEQ ID NO: 6). An equivalent independently isolated product with a different mutation but equivalent properties is designated REM-T5 (SEQ ID NO: 8).

15

20

In addition to the modifications of native *Taq* DNA polymerase present in the modified *Taq* DNA polymerase of the present invention, individual amino acid residues in the peptide chain comprising the *Taq* DNA polymerase may be modified or deleted without eliminating any of the requisite properties described herein. Such alterations that do not destroy activity do not remove the DNA sequence or the modified *Taq* DNA polymerase from the contemplated scope of the present invention.

10

15

20

In order to assay the modified Taq DNA polymerase, REM-T3 (SEQ ID NO: 6), it was necessary to isolate it. We used the following novel, short isolation technique producing high purity enzyme quickly. Bacteria were grown overnight or to an OD at 600 nm of about 2.0 to 2.5 and then centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet washed with a solution of 50 mM Tris(8.0), 50 M dextrose, and 1 mM EDTA (15 x cell wt). The pellet was re suspended and lysed with a solution of 50 mM Tris, 50 mM dextrose, 1 mM EDTA. and 1 mg/ml lysozyme(5x cell wt). An equal volume of a solution of 10 mM Tris and 50 mM KCl, and 1 mM EDTA was added and the resulting mixture incubated at 75°C for 60 min before centrifuging at 8000 rpm for 15 min. The pellet was discarded and an equal volume of DEAE and 0.4 M KPO4 (6.8) was added to the supernatant. The mixture was then incubated at 0°C for 30 min and then centrifuged at 10,000 rpm for 20 min. The pellet was discarded and the supernatant put on a phosphocellulose column with 0.02 M KPO₄ (7.5)(4x cell wt). The column was eluted with a gradient of 0.02 to 0.4 M KPO₄ (7.5). The peak was collected and applied to a Bio Rex-70 column with a solution of 0.02 M KPO₄ (7.6), 80 mM KCl 5%, glycerol, 0.5% Tween, and 0.5% Nonidet P-40. This column was then eluted with a step gradient of 0.3 M KCl and the peak collected.

The thermostability of the modified *Taq* DNA polymerase of the present invention must be substantially equivalent to that of native *Taq* DNA polymerase, i.e., it must not become irreversibly denatured (inactivated) when subjected to the elevated temperatures for the time necessary to effect denaturation of double-stranded nucleic acids. The heating conditions (e.g., temperature and time) necessary

10

15

20

for denaturation will depend on a variety of factors, including the buffer salt concentration and the length and composition of the nucleotide chain. Typically, the temperature range for which the enzyme must be stable is about 90 to about 105°C for about 0.5 to four minutes. These values may vary depending on the conditions.

The modified *Taq* DNA polymerase of the present invention preferably functions optimally at temperatures above 40°C. The enzymes of the present invention is active in the temperature range 55 - 95°C, and preferably in the range 70 - 95°C.

U.S. Pat. No. 4,889,818 discloses and claims a native form of *Taq* DNA polymerase. Because the modified *Taq* DNA polymerase of the present invention retains all the characteristics of the native form that are useful in PCR technology, its use in PCR is preferable to the native form. Consequently, applications using *Taq* DNA polymerase as described in U.S. Pat. No. 4,889,818, col. 14, 1. 33 to col. 27, l. 27 may also use the modified *Taq* DNA polymerase of the present invention. Accordingly, the disclosure of U.S. Pat. No. 4,889,818 is hereby incorporated by reference.

Besides use in the polymerase chain reaction, the modified *Taq* DNA polymerase of the present invention can be used in DNA sequencing by, for example, the Sanger dideoxy-mediated chain-termination method. Sanger et al., *Proc. Natl. Acad. Sci.* 74, 5463 (1977). Other similar uses will be known to those of skill in the art.

The following examples further elucidate the present invention, but are not intended to limit it.

WO 95/14782 PCT/US94/13554

16

EXAMPLE 1

Zone Mutagenesis of the Taq DNA Polymerase Gene - Treatment 1

5

10

15

20

The *Taq* polymerase gene was amplified from genomic DNA (*Thermus aquaticus*) using primers adding an EcoRI site in the 5° UTR (nucleotide 70) and BgII site at the 3° end (nucleotide 2619). The the PCR product was cloned into pUC18 after digesting the vector with EcoRI and BamHI. *See* Figure 1. We designated this *Taq* gene REM-T2. We then incubated the plasmid containing the *Taq* gene at pH 4.8 (10 mM sodium acetate) and room temperature for 20 minutes followed by neutralization to pH 8.0 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)) followed by digestion with Eco RI and KpnI.

The pLSM5 (SEQ ID NO: 3) vector was digested with EcoRI and KpnI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5 '-3' exonuclease activity.

EXAMPLE 2

Zone Mutagenesis of the Taq DNA Polymerase Gene - Treatment 2

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. See Figure 1. We incubated the plasmid DNA containing the

10

15

20

Taq gene at pH 4.8 and 60°C for 5 minutes followed by neutralization to pH 8.0 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11)) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)) followed by digestion with Eco RI and KpnI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and KpnI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5 '-3' exonuclease activity.

EXAMPLE 3

Zone Mutagenesis of the Taq DNA Polymerase Gene - Treatment 3

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. *See* Figure 1. We amplified the N-terminal region of the *Taq* DNA polymerase gene for three consecutive PCR programs of 30 cycles each using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)). Inserts for the putative amino terminal region of the gene were generated by digestion of the PCR products with Eco RI and KpnI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and KpnI and purified.

The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5 '-3' exonuclease activity.

5

EXAMPLE 4

Zone Mutagenesis of the Taq DNA Polymerase Gene - Treatment 4

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. *See* Figure 1. We incubated the plasmid DNA containing the *Taq* gene a pH 4.8 and 70°C for 15 minutes followed by neutralization to pH 8 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11)) and the "sequencing primer" 1155A (CAG GTC CCT GAG GGC (SEQ ID NO: 13)) and 5x concentration of dNTPs (0.75 mM) followed by digestion with Eco RI and BstXI.

15

20

10

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and BstXI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5 '-3' exonuclease activity.

EXAMPLE 5

DNA Polymerase Activity Assay

Assay mixture:

reaction volume: 0.3 ml

25 mM Tris-HCl (pH = 8.8)

4 mM MgCl₂

22 µg activated ssDNA (salmon sperm)

0.033 mM dNTP (each)

2 μ Ci [methyl-3H] thymidine 5 triphosphate

10 enzyme

5

15

Assay procedure:

The mixture was incubated at 75°C for 10 minutes. The reaction was stopped with 2 ml ice cold 10% TCA - 0.1 M sodium pyrophosphate. The tubes were then placed on ice for 10 minutes and the reaction volume filtered. The tube and filter were washed three times with 2 ml of 10% TCA - 0.1 M sodium pyrophosphate. The filter was then washed with 10 ml 0.01 N HCl. Next the filters were dried at 120°C for 15 minutes. The dried filters were counted in 1 ml of Scintiverse.

The results are displayed in Table 1, infra.

PCT/US94/13554

5

10

15

20

20

EXAMPLE 6

5'-3' Exonuclease Activity Assay

Preparation of double stranded substrate with blunt ends and removal of 5 phosphate

A Blue-Script plasmid was cut with HincII to produce one double stranded piece with blunt ends and treated with CIP (calf intestine phosphatase) to remove the 5 phosphate.

End-labeling of the 5 ends using [0-\ o(.32)P]ATP

8 μ l plasmid and 4 μ l buffer were mixed with spermidine and 28 μ l distilled H₂O. The mixture was then heated to 70°C for 5 minutes and then chilled on ice for 2 minutes. 10 μ l kinase buffer with 1 μ l [g-32P]ATP (about 10 mCi) and 2 μ l (20 units) of T4 polynucleotide kinase were added. Then the mixture was incubated for 30 minutes at 37°C. The reaction was stopped by adding 2 μ l 0.5 M EDTA. The enzyme was inactivated by incubating for 10 minutes at 70°C. The radioactive ATP was removed by washing 4 times (2 ml each) in Centricon 100. The final volume was about 50 μ l (38,000 cpm/ μ l).

5'-3' exonuclease assay Assay conditions:

reaction volume 50 μ l 25 mM Tris HCl (8.8)

4 mM Mg Cl,

 $0.5 - 1 \mu l$ labeled substrate

0.3 units of DNA polymerase

Samples were incubated at 50 - 55°C for 15, 30 or 60 minutes. The reaction was stopped with 0.3 ml 10% TCA. The sample was microfuged for 15 minutes at 4°C. 0.1 ml was sampled on filter paper. The filter paper was dried at 120°C for 15 minutes. Dried filters were counted in 1 ml of Scintiverse.

The assay results are presented in Table 1, infra.

EXAMPLE 7

10

15

20

5

Sequencing Mutant Genes

Three mutants were chosen from those listed in Table 1 for low exonuclease activity. These were colony 18' (the plasmid of which we designate pTarf2 (SEQ ID NO: 9)) and colony 20' (the plasmid of which we designate pTarf3 (SEQ ID NO: 5)). A third mutant, pTarf5 (SEQ ID NO: 7), was obtained in a similar manner as in Example 4. pTarf3 (SEQ ID NO: 5) produces REM-T3 (SEQ ID NO: 6) and pTarf5 (SEQ ID NO: 7) produces REM-T5 (SEQ ID NO: 8). Bi-directional sequencing of the nucleic acid sequence of these mutants was conducted in the following manner: DNA sequence analysis was performed on alkaline-denatured double stranded plasmids. We used synthesized oligonucleotide primers (Fig. 3), [α-stS]-dATP, and Sequenase® T7 DNA polymerase kit (United States Biochemical Corp.) according to the manufacturer's conditions. This method is based on the dideoxy chain termination reaction (Sanger, Science 214, 1205 (1981).

The alterations found in the mutants are presented in Table 2. These alterations are of the pLSM5 (SEQ ID NO: 3) sequence, i.e., the pTarf2 (SEQ ID NO: 9), pTarf3 (SEQ ID NO: 5), and pTarf5 (SEQ ID NO: 7) sequences are the same as the pLSM5 (SEQ ID NO: 3) sequence except for the alterations listed in Table 2.

TABLE 1 Enzyme Activity Of New Taq Clones

10	treatment	colony	polymerase act units/μl	5'-3' exonuclease activity % of REM-T2 (SEQ ID NO: 4)
*	1	1	0.132	87
-		2	0.503	97
		3	0.053	14
;		4	0.27	88
15		5	0.098	82
		6	0.41	94
		7	0.255	95
,	2	8	0.106	74
	1	1.	1.54	104
20		2 .	1.60	94
		3 .	1.06	105
		4 '	1.49	-100
		5 '	1.06	104
		6.	2.20	114
25		7.	0.35	107
!		8.	0.68	117

	9.	0.74	94
	10 ′	0.87	109
2	11.	1.81	98
	12.	1.22	95.
	13 '	1.68	110
	14 .	1.04	102
	15 -	0.84	101
1.4.	16	1.4	98
	17	0.15	104
	18	1.77	. 24
	19 .	1.11	107
3	20 ·	1.73	0
	21 '	0.018	6
	22 ·	0.48	0
	23 ·	1.8	105
	24	0.83	94
	25	0.78	93

1 unit of polymerase activity = 10 nmoles of total nucleotides incorporated into acid insoluble form in 30 minutes at 75°C. Primed and unprimed colonies were obtained from cells transformed on different days.

WO 95/14782 PCT/US94/1355-

TABLE 2

Alterations Relative to pLSM5 (SEQ ID NO: 3)

plasmid	nucleotide position	amino acid position	codon change	amino acid change
p T a r f 2 (SEQ ID NO: 9)	337	73	ттс-стс	Phe-Leu
pTarf3	193	25	CGC-TGC	Arg-Cys
(SEQ ID NO: 5)	504	128	AAG-AAA	Lys-Lys
p T a r f 5 (SEQ ID NO: 7)	341	74	CGC-CAC	Arg-His

15

10

5

EXAMPLE 8

Improved Processivity of the Modified Taq Polymerase

Processivity of DNA synthesis by the modified Taq DNA polymerase (REM-T3) was assessed by several trials, with comparison to commercial enzymes and REM-T2. The method using the PCR protocol is novel.

20

25

Trial 1: Gel analysis of processivity by thermal stable DNA polymerases.

M13mp18 template (0.25 pmol/10 μ l) and 5 ³²P-labeled 17-mer (M13/pUC-40, BioLabs) (0.50 pmol/10 μ l) (calculated $t_m = 52^{\circ}$ C) were annealed in 40 μ l of 10 mM Tris-HCl (pH 8.0), and 5 mM MgCl₂. The mixture was incubated for 3 minutes at 90°C, 20 minutes at 42°C, and 15 minutes at room temperature. The reaction mixture was adjusted to 200 μ M each of dNTP, 0.05% Tween 20 and Nonidet P-40, 10 mM Tris-HCl (pH 8.0), 50 mM KCl and 2.5 mM MgCl₂, in a total

10

15

20

volume of 80 μ l, then incubated at 55°C for 2 minutes without enzyme. Next, 0.94 units of enzyme (AmpliTaqTM (Cetus), Stoffel Fragment(Cetus), REM-T2 or REM-T3)/10 μ l were added to start the reaction. Five μ l aliquiots were removed from the reaction mixture at 0, 15, 30, 45 seconds, and 1, 2, and 5 minutes and added to 5 μ l of stop solution (1 mg/ml each of xylene cyanol and bromphenol blue, 10 mM EDTA in formamide). For gel analysis, 5 μ l were loaded onto a 6% wedge acrylamide/urea gel.

Figure 4 is a schematic depiction of the process and Figure 5 is an autoradiograph showing the results of trial 1.

Trial 2: Gel analysis of processivity by thermal stable DNA polymerases.

The same method was used as in Trial 1, except 0.22 units of polymerase/10 μ l of reaction mixture were added. In addition, smaller volumes were used for annealing (25 μ l) and reaction mixture (50 μ l).

For trials 1 and 2, the assayed polymerase activity of the AmpliTaq[™] was lower than usual. It appears from the gels that the number of actual units of AmpliTaq[™] used in the reaction may have been higher that estimated and, therefore, may not be comparable to the other reactions.

Figure 6 shows the results of trial 2. Note that when the amount of polymerase is limiting, REM-T2 (SEQ ID NO: 4) and REM-T3 (SEQ ID NO: 6) have processivities greater than that of the Stoffel fragment.

WO 95/14782 PCT/US94/13554

26

Trial 3: PCR analysis of processitivity by thermal stable DNA polymerases.

The final volume of PCR reaction was 50 μ l. The buffer contained 67 mM Tris-HCI (pH 8.8), 16 mM (NH₄)₂SO₄, 10 mM beta mercaptoethanol, 2 mM MgCl₂, 6.7 μ M EDTA, and 150 μ M each dNTP. There was an excess of template (0.02 pmol/10 μ l) and primers (each 10 pmol/10 μ l) over enzyme (0.04 units of polymerase/ 10 μ l) for each PCR reaction. The template was pLSM5 (SEQ ID NO: 3), a 5.1 kb plasmid containing Taq DNA polymerase gene and used for sequencing. For the 834-951 primer set, at least 102 nucleotides must be added to the primers to form the 117 base pair product, and for the 1564-1937 primer set, at least 358 nucleotides must be added to the primer to form the 373 base pair product. The PCR program was 20 sec denaturation at 94°C, 30 sec annealing at 48°C, and 2 min extension at 72°C for 12 cycles.

Figure 7 is a schematic depiction of this process and Figure 8 shows is an autoradiograph showing the results.

15

20

10

5

Interpretation of Processivity Testing

Trials I and 2 are based on methodology similar to Innis et al., *Proc. Natl. Acad. Sci.* 85, 9436 (1988); Tabor et al., *J. Biol. Chem.* 262, 16212 (1987); and Wernette et al., *Biochem.* 27, 6046 (1988). The use of a fixed primer for synthesis under conditions of limiting enzyme activity and excess template/primer allows analysis of the length of extension of the primer with minimal chance for re-initiation. Thus, analysis of product size by polyacrylamide/urea gel measures primer extension as a unit event, or processivity of the polymerase (trials 1 and 2).

10

15

20

Trial 3 is based on a new approach. We reasoned that it would be possible to measure processivity under conditions of PCR. With limiting enzyme concentration and excess primer/template concentration, the probability of re-initiation on a partially extended primer in PCR cycles is very low. Therefore, the length of the observed product (resulting from the complete extension of a primer through the opposing primer) is a measure of processivity. We found that 12 cycles results in sufficient yield to detect products with ethidium bromide on agarose gel. By varying the distance between primers we can determine a processivity range. AmpliTaq REM-T2, and REM-T3 have a processivity of at least 105 nucleotides, but less that 358 nucleotides. Stoffel Fragment, on the other hand has a processivity of less than 105 nucleotides.

Figure 8 compares the ability of four polymerases to extend a primer 105 nucleotides (Lanes 1-4) or 358 nucleotides (Lanes 5-8) under PCR conditions of excess DNA template (0.02 pmol/10 μ l of reaction) and primer (10 pmol/10 μ l of reaction) and limited polymerase units (0.04 units of polymerase/10 μ l reaction). PCR products are shown on a 3% NuSieve gel. AmpliTaqTM is in lanes 1 and 5, Stoffel Fragment is in lanes 2 and 6, REM-T2 in lanes 3 and 7, and REM-T3 in lanes 4 and 8. Marker lane has ϕ x 174/Hae III.

It is evident from an examination of Figures 6, 7, and 8 that REM-T3 (SEQ ID NO: 6) has a processivity equal to or better than AmpliTaq[™], and much better than the Stoffel fragment. This result demonstrates that the full length polypeptide of the modified *Taq* enzyme confers superior processivity compared to the truncated peptide of the Stoffel enzyme.

WO 95/14782 PCT/US94/13554

28

EXAMPLE 9

Misincorporation Rate for Modified Taq DNA Polymerases

Information already published by Barnes, Gene 112, 29-35 (1992) indicates that Taq DNA polymerase which has had the N-terminal region containing the 5' exonuclease domain removed has a diminished misincorporation rate. The information available indicates that such a modified Taq DNA polymerase has a two-fold lower misincorporation rate than native Taq DNA polymerase. Since the evidence presented by Barnes leads to the conclusion that the misincorporation by the Taq DNA polymerase is lowered in the absence of the exonuclease activity, we are motivated to measure the misincorporation rate of the modified Taq DNA polymerases described herein.

5

10

15

20

The assessment of misincorporation is done by several methodologies:

- 1. The methodology of Barnes uses a specially constructed plasmed with a flanking selectable marker, based on identification of *lacZ* as an indicator gene. Scoring for misincorporation in the *lac* gene is by the familiar blue/white test on an indicator dye (XGal). Testing for misincorporation is performed by inserting the plasmids into an indicator bacterial strain following PCR reactions *in vitro*.
- 2. The methodology of Tindall and Kunkel, *Biochemistry* 21, 6008-6013 (1988) monitors the fidelity of *in vitro* DNA synthesis using the *lacZ* gene for a complementation in a plasmid derived from M13 bacteriophage. Measurement of misincorporation is based on the blue/white test for *lacZ* function using an indicator dye in the plate. The plasmid derivative contains an open single-stranded gap region

10

of 390 nucleotides. This construction allows measurement of the forward mutation rate, or the substantially lower reversion mutation rate for any specific misincorporation constructed. The results found by Kunkel and coworkers, indicate that the native *Taq* DNA polymerase has a base substitution error rate of approximately 1/9000 nucleotides polymerized.

The processivity of our modified *Taq* DNA polymerase is much higher than the processivity of the truncated proteolytic fragment, and since the DNA polymerase literature indicates that misincorporation correlates with re-initiation, our misincorporation rate is considerably improved relative to native *Taq* DNA polymerase.

SEQUENCE LISTING

5	(1) GENERAL INFORMATION:
ر	(i) APPLICANT: Moses M.D., Robb E.
10	(ii) TITLE OF INVENTION: Modified Thermo-Resistant DNA Polymerases
10	(iii) NUMBER OF SEQUENCES: 15
15	(iv) CORRESPONDENCE ADDRESS: (A) ADDRESSEE: Allegretti & Witcoff (B) STREET: 10 South Wacker Drive (C) CITY: Chicago (D) STATE: IL (E) COUNTRY: USA
20	(F) ZIP: 60606
25	 (v) COMPUTER READABLE FORM: (A) MEDIUM TYPE: Floppy disk (B) COMPUTER: IBM PC compatible (C) OPERATING SYSTEM: FC-DOS/MS-DOS (D) SOFTWARE: Patentin Release \$1.0, Version \$1.25
30	(vi) CURRENT APPLICATION DATA: (A) APPLICATION NUMBER: (B) FILING DATE: (C) CLASSIFICATION:
35	(viii) ATTORNEY/AGENT INFORMATION: (A) NAME: Greenfield Ph.D., Michael S. (B) REGISTRATION NUMBER: 37,142 (C) REFERENCE/DOCKET NUMBER: 93,413
40	(ix) TELECOMMUNICATION INFORMATION: (A) TELEPHONE: (312)715-1000 (B) TELEFAX: (312)715-1234
	(2) INFORMATION FOR SEQ ID NO::1:
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 2626 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
50	(ii) MOLECULE TYPE: cDNA
	(iii) HYPOTHETICAL: NO
55	(iv) ANTI-SENSE: NO
,,	(vi) ORIGINAL SOURCE: (A) ORGANISM: Thermus aquaticus
60	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1212619
	(ix) FEATURE:

				(A) (B)						le';	٠			11	. •		
5		(i :		EATU (A) 1 (B) 1 (D) (NAME, LOCA: OTHE	rion Rini	: 1. FORM	ATIO:	N: /	note	= "N	ativ	е Та	g DN	'A Po	lymera	se
10	-	(x:	L) SI	EQUEN	ICE I	ESCI	RIPT	ON:	SEQ	ID 1	10::	1:					
	AAG	CTC	AGAT	CTAC	CTGC	CT G	AGGG	CGT	C GC	TTCC	AGC:	r GG(CCT	rccc	GAG	GGGAGA	60
15	GGG	GAGGG	GTT	TCTA	AAAG	cc c	TTCA	GGAC	x ငာ	ACCO	xccc	GCC	GGT	GTG	GAAC	GGTAAC	120
20	ATC Met	Arc	GGG Gly	ATG Met	CTG Leu	Pro	CTC Leu	TTT Phe	GAG Glu	CCC Pro	Ly	GGC GGC	CGG	GTC Va	CTC Le	CTG u Leu 5	168
20	GTC Val	GAC Asp	GGC Gly	CAC His	HTB	CTG Leu	GCC Ala	TAC	CGC Arg	, Thr	TTC Phe	CAC Hie	GCC Ala	CTG Let	Ly:	GGC Gly	216
25	CTC Leu	ACC Thr	ACC Thr	ser	CGG Arg	GGG Gly	GAG Glu	CCG Pro 40	val	CAG Gln	GCG Ala	GTC Val	TAC Tyr	Gl	TTC Phe	GCC Ala	264
30	AAG Lys	AGC Ser 50	Leu	CTC Leu	AAG Lys	GCC Ala	CTC Leu 55	Lys	GAG Glu	GAC Asp	GGG Gly	GAC Asp 60) Ala	GTG Val	ATC Ile	GTG Val	312
35	GTC Val 65	TTT Phe	GAC Asp	GCC Ala	AAG Lys	GCC Ala 70	CCC Pro	TCC Ser	TTC Phe	CGC Arg	CAC His 75	Glu	GCC Ala	TAC Tyr	GGG Gly	GGG Gly 80	360
40	TAC Tyr	AAG Lys	GCG Ala	GGC Gly	CGG Arg 85	GCC Ala	CCC Pro	ACG Thr	CCG Pro	GAG Glu 90	GAC Asp	TTT Phe	CCC Pro	CGG Arg	CAA Gln 95	Leu	408
	GCC Ala	CTC Leu	ATC Ile	AAG Lys 100	GAG Glu	CTG Leu	GTG Val	GAC Asp	CTC Leu 105	CTG Leu	GGG Gly	CTG Leu	GCG Ala	CGC Arg 110	Leu	GAG Glu	456
45	GTC Val	CCG Pro	GGC Gly 115	TAC Tyr	GAG Glu	GCG Ala	GAC Asp	GAC Asp 120	GTC Val	CTG Leu	GCC Ala	AGC Ser	CTG Leu 125	GCC Ala	AAG Lys	AAG Lys	504
50	GCG Ala	GAA Glu 130	AAG Lys	GAG Glu	GGC Gly	TAC Tyr	GAG Glu 135	GTC Val	CGC Arg	ATC Ile	CTC Leu	ACC Thr 140	GCC Ala	GAC Asp	AAA Lys	GAC Asp	552
55	CTT Leu 145	TAC Tyr	CAG Gln	CTC Leu	CTT Leu	TCC Ser 150	GAC Asp	CGC Arg	ATC Ile	CAC His	GTC Val 155	CTC Leu	CAC His	CCC Pro	GAG Glu	GGG Gly 160	600
50	TAC Tyr	CTC Leu	ATC Ile	ACC Thr	CCG Pro 165	GCC Ala	TGG Trp	CTT Leu	TGG Trp	GAA Glu 170	AA Lys	TAC Tyr	GGC Gly	CTG Leu	AGG Arg 175	CCC Pro	648
-	GAC Asp	CAG Gln	TGG Trp	GCC Ala 180	GAC Asp	TAC Tyr	CGG Arg	GCC Ala	CTG Leu 185	ACC (GGG GLy	GAC Asp	GAG Glu	TCC Ser 190	GAC Asp	AAC Asn	696

	CT: Lev	r ccc	GGG Gly 195	' Vai	L AAG	GGC Gly	ATC	GGG Gly 200	A CT	AAG 1 Lyi	ACG Thi	GCG F Ala	AGG Arg	J Ly	CT B Le	r CTG u Leu	744
5	GA0 Glu	GAG Glu 210	Trp	GGG Gly	AGC Ser	CTG Leu	GAR Glu 215	ı Ala	CTC Leu	CTC Leu	AAG Lys	AAC ABI 220	ı Leı	GAC 1 As	CGC Ar	CTG g Leu	792
10	AAG Lys 225	Pro	GCC Ala	ATC	CGG Arg	GAG Glu 230	Lye	ATC Ile	CTG Leu	GCC Ala	CAC His 235	: Met	GAC Asp	GAT Asi	CTC Le	AAG Lys 240	840
15	CTC	TCC Ser	TGG	GAC Asp	CTG Leu 245	Ala	AAG Lys	GTG Val	CGC Arg	ACC Thr 250	Asp	CTG Leu	CCC Pro	CTG Leu	GAG Glu 255	GTG Val	888
20	Asp	Phe	Ala	Lys 260		Arg	Glu	Pro	265	Arg	Glu	Arg	Leu	Arg 270	Ala	Phe	936
	CTG Leu	GAG Glu	AGG Arg 275	CTT Leu	GAG Glu	TTT Phe	GGC Gly	AGC Ser 280	Leu	ĊTC Leu	CAC His	GAG Glu	TTC Phe 285	GGC Gly	CTT	CTG Leu	984
25	GAA Glu	AGC Ser 290	Pro	AAG Lys	GCC Ala	CTG Leu	GAG Glu 295	GAG Glu	GCC Ala	CCC Pro	TGG Trp	CCC Pro 300	CCG Pro	CCG Pro	GAA Glu	GGG Gly	1032
30	GCC Ala 305	Phe	GTG Val	GGC Gly	TTT Phe	GTG Val 310	CTT Leu	TCC Ser	CGC Arg	AAG Lys	GAG Glu 315	CCC Pro	ATG Met	TGG Trp	GCC Ala	GAT Asp 320	1080
35	CTT Leu	CTG Leu	GCC Ala	Leu	GCC Ala 325	GCC Ala	GCC Ala	AGG Arg	GGG Gly	GGC Gly 330	CGG Arg	GTC Val	CAC His	CGG Arg	GCC Ala 335	CCC Pro	1128
40	GAG Glu	CCT Pro	TAT Tyr	AAA Lys 340	GCC Ala	CTC Leu	AGG Arg	GAC Asp	CTG Leu 345	AAG Lys	GAG Glu	GCG Ala	CGG Arg	GGG Gly 350	CTT Leu	CTC	1176
	GCC Ala	AAA Lys	GAC Asp 355	CTG Leu	AGC Ser	GTT Val	CTG Leu	GCC Ala 360	CTG Leu	AGG Arg	GAA Glu	GGC Gly	CTT (Leu 365	GGC Gly	CTC Leu	CCG Pro	1224
45	CCC Pro	GGC Gly 370	GAC Asp	GAC Asp	CCC Pro	Met	CTC Leu 375	CTC Leu	GCC Ala	TAC Tyr	CTC Leu	CTG Leu 380	GAC (Asp	CCT '	TCC Ser	AAC Asn	1272
50	ACC Thr 385	ACC Thr	CCC Pro	GAG Glu	GGG G1y	GTG Val 390	GCC Ala	CGG Arg	CGC '	TAC (GGC (Gly 395	GGG (Gly	GAG : Glu	TGG :	ACG Thr	GAG Glu 400	1320
55	GAG Glu	GCG Ala	GGG Gly	GAG Glu	CGG Arg 405	GCC (GCC Ala	CTT : Leu	Ser	GAG A Glu 410	AGG (Arg	CTC : Leu	TTC (Phe	GCC A	AAC Asn 415	CTG Leu	1368
60	TGG Trp	G1A GGG	AGG Arg	CTT Leu 420	GAG Glu	GGG (Gly	GAG (Glu	Glu	AGG (Arg 425	CTC (Leu	CTT :	Trp	Leu	TAC (Tyr 430	CGG (Arg	GAG Glu	1416
00	GTG Val	GAG Glu	AGG Arg 435	CCC Pro	CTT Leu	TCC (Ser	Ala	GTC (Val 440	CTG (Leu :	GCC (CAC A	Met	GAG C	CC I	ACG (Thr	GGG Gly	1464

	G: Va:	rg co l Arg 450	Lec	G GA ABP	C GT Val	G GCO	Tyr 455	Let	C AGG	G GCG Ala	C TTO	G TC	r Le	G GA	G GTY	G GCC l Ala	1512
5	GA0 Glv 465	1 GIL	ATC 111e	GCC Ala	CGC Arg	CTC Leu 470	Glu	GCC Ala	GAG Glu	GTC Val	TTC Phe 475	: Arq	CTG Lei	GCC Al	GGC a Gl	CAC Y His 480	1560
10	Pro	TTC Phe	AAC Asn	CTC Leu	AAC Asn 485	TCC	CGG Arg	GAC Asp	CAG Gln	CTG Leu 490	Glu	AGG Arg	GTC Val	CTC Le	TTT Phe 495	Asp	1608
15	GAC Glu	CTA Leu	GGG Gly	CTT Leu 500	Pro	GCC Ala	ATC Ile	GCC	AAG Lys 505	Thr	GAG Glu	AAG Lys	ACC Thr	GGC Gl ₃ 510	Lys	CGC Arg	1656
20	TCC Ser	ACC Thr	AGC Ser 515	GCC Ala	GCC Ala	GTC Val	CTG Leu	GAG Glu 520	Ala	CTC Leu	CGC	GAG Glu	GCC Ala 525	His	CCC Pro	ATC Ile	1704
20	GTG Val	GAG Glu 530	Lys	ATC	CTG	CAG Gln	TAC Tyr 535	CGG Arg	GAG Glu	CTC Leu	ACC	AAG Lys 540	Leu	AAG Lys	AGC Ser	ACC Thr	1752
25	TAC Tyr 545	Ile	GAC Asp	CCC Pro	TTG Leu	CCG Pro 550	GAC Asp	CTC Leu	ATC Ile	CAC His	CCC Pro 555	AGG Arg	ACG Thr	GGC Gly	CGC Arg	CTC Leu 560	1800
30	CAC	ACC	CGC Arg	TTC Phe	AAC Asn 565	CAG Gln	ACG Thr	GCC Ala	ACG Thr	GCC Ala 570	ACG Thr	GGC Gly	AGG Arg	CTA Leu	AGT Ser 575	AGC Ser	1848
35	TCC Ser	GAT Asp	CCC	AAC Asn 580	CTC Leu	CAG Gln	AAC Asn	ATC Ile	CCC Pro 585	GTC Val	CGC Arg	ACC Thr	CCG Pro	CTT Leu 590	Gly	CAG Gln	1896
40	AGG Arg	ATC	CGC Arg 595	CGG Arg	GCC Ala	TTC Phe	ATC Ile	GCC Ala 600	GAG Glu	GAG Glu	GGG Gly	TGG Trp	CTA Leu 605	TTG Leu	GTG Val	GCC Ala	1944
	CTG Leu	GAC Asp 610	Tyr	AGC Ser	CAG Gln	ATA Ile	GAG Glu 615	CTC Leu	AGG Arg	GTG Val	CTG Leu	GCC Ala 620	CAC His	CTC Leu	TCC Ser	GGC Gly	1992
45	GAC Asp 625	GAG Glu	AAC Asn	CTG Leu	ATC Ile	CGG Arg 630	GTC Val	TTC Phe	CAG Gln	GAG Glu	GGG Gly 635	CGG Arg	GAC Asp	ATC Ile	CAC His	ACG Thr 640	2040
50	GAG Glu	ACC Thr	GCC Ala	AGC Ser	TGG Trp 645	ATG Met	TTC Phe	GGC Gly	GTC Val	CCC Pro 650	CGG Arg	GAG Glu	GCC Ala	GTG Val	GAC Asp 655	CCC Pro	2088
55	CTG Leu	ATG Met	CGC Arg	CGG Arg 660	GCG Ala	GCC . Ala	AAG Lys	ACC Thr	ATC . Ile 665	AAC Asn	TTC Phe	GGG Gly	GTC Val	CTC Leu 670	TAC Tyr	GGC Gly	2136
	ATG Met	TCG Ser	GCC Ala 675	CAC His	CGC Arg	CTC Leu	TCC Ser	CAG Gln 680	GAG Glu	CTA (Leu	GCC Ala	ATC Ile	CCT Pro 685	TAC Tyr	GAG Glu	GAG Glu	2184
60	Ala	CAG Gln	Ala	TTC Phe	ATT Ile	Glu	CGC Arg	TAC Tyr	TTT Phe	CAG . Gln	Ser	TTC Phe	CCC Pro	AAG Lys	GTG Val	CGG Arg	2232

5	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val 705 710 715 720	280
,	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG Glu Thr Leu Phe Gly Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 725 730 735	328
10	GTG AAG AGC GTG CGG GAG GCC GCC GAG CGC ATG GCC TTC AAC ATG CCC Val Lys Ser Val Arg Glu Ala Ala Glu Arg Het Ala Phe Asn Met Pro 740 745 750	376
15	GTC CAG GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC 24 Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu 755 760 765	24
20	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 770 780	72
25	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 795 800	20
	CGG CTG GCC AAG GAG GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro 805 815	68
30	CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG . 26: Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 830	16
35	TGATACCACC 26	26
	(2) INFORMATION FOR SEQ ID NO::2:	
40	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 832 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear	
45	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::2:	
50	Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 10 15	
	Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 20 25 30	
55	Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	
	Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60	
60	Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly 65 70 75 80	

	Туг	Lys	Ala	Gly	. Arg 85	Ala	Pro	Thr	Pro	90	Asp	Phe	Pro	Ar	g G1: 9:	n Leu 5
5	Ala	Leu	Ile	Lys 100	Glu	Leu	Val	Asp	105	Leu	Gly	Leu	Ala	Arg 110	J Le	ı Glu
			115					120					125	•		3 Lys
10		130					135					140				Asp
15	143					150					155					160
					165					170					175	Pro
20				180	Asp				185					190		
			19	5	Lys			200)				20	5		
25		210			Ser		215					220				
30	225	: :			Arg	230					235		•			240
	Leu				245					250					255	
35				260	Arg			•	265					270		
40			2/5		Glu			280					285			
		290			Ala		295					300				-
45	305				Phe	310					315					320
					Ala 325					330				_	335	
50				340	Ala				345					350		
55			355		Ser			360					365			
		3/0			Pro		375					380				
60	385					390					Gly 395					400
	Glu	utg	тÀ	GIU	Arg . 405	Ala .	WTS :	Leu :	ser	Glu . 410	Arg :	Leu~l	Phe .		Asn 415	Leu

	Trp	Gly	Arg	Leu 420	Glu	Gly	Glu	Glu	Arg 425	Leu	Leu	Trp	Leu	Tyr 430	Arg	Glu
5	Val	Glu	Arg 435	Pro	Leu	Ser	Ala	Val 440	Leu	Ala	His	Met	Glu 445	Ala	Thr	Gly
	Val	Arg 450	Leu	Asp	Val	Ala	Tyr 455	Leu	Arg	Ala	Leu	Ser 460	Leu	Glu	val	Ala
10	Glu 465	Glu	Ile	Ala	Arg	Leu 470	Glu	Ala	Glu	Val	Phe 475	Arg	Leu	Ala	Gly	His 480
15	Pro	Phe	Asn	Leu	Asn 485	Ser	Arg	Asp	Gln	Leu 490	Glu	Arg	Val	Leu	Phe 495	Asp
	Glu	Leu	Gly	Leu 500	Pro	Ala	Ile	Gly	Lys 505		Glu		Thr	Gly 510	Lys	Arg
20	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala				Ala 525	His	Pro	Ile
	Val	Glu 530	Lys	Ile	Leu	Gln	Tyr 535	Arg	Glu	Leu	Thr	Lys 540	Leu	Lys	Ser	Thr
25	Tyr 545	Ile	Asp	Pro	Leu	Pro 550	Asp	Leu	Ile	His	Pro 555	Arg	Thr	Gly	Arg	Leu 560
30	His	Thr	Arg	Phe	Asn 565	Gln	Thr	Ala	Thr	Ala 570	Thr	Gly	Arg	Leu	Ser 575	Ser
	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Leu 590	Gly	Gln
35			595	_			Ile	600					605			
		610	-				Glu 615					620				
40	625					630	Val				635					640
45	Glu	Thr	Ala	Ser	Trp 645	Ket	Phe	Gly	Val	Pro 650	Arg	Glu	Ala	Val	Asp 655	Pro
			Ī	660			Lys		665					670		
50			675				Ser	680					685			
		690					Arg 695					700				
55	705					710	Leu				715					720
60					725		Arg			730					735	
••	Val	Lys		Val 740		Glu	Ala	Ala	Glu 745	Arg	Met	Ala	Phe	Asn 750	Met	Pro

	Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu 755 760 765
5	Phe Pro Arg Leu Glu Glu Het Gly Ala Arg Met Leu Leu Gln Val His 770 780
	Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 790 795 800
10	Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro 805 810 815
15	Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 830
	(2) INFORMATION FOR SEQ ID NO::3:
20	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 2626 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
25	(ii) MOLECULE TYPE: DNA (genomic)
	(iii) HYPOTHETICAL: NO
30	(iv) ANTI-SENSE: NO
	(vi) ORIGINAL SOURCE: (A) ORGANISM: Thermus aquaticus
35	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(89, "g") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at postion 89 of the native Tag DN.</pre>
40	polymerase nucleofide sequence of C to G."
45	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(934, "a") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native Tag DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."</pre>
50	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(962, "c") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native Tag</pre>
55	DNA polymerase nuclectide sequence of T to C. This results in an amino acid change of Leu to Pro.
60	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(2535, "a") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 2535 of the native Tag DNA polymerase nucleotide sequence of G to A. This mutation is conservative."</pre>

38.

	(ix) FEATURE:	
	(A) NAME/KEY: CDS (B) LOCATION: 1212619	
5	(ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 1212616	
10	(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 12619 (D) OTHER INFORMATION: /note= "pLSM5"	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::3:	
	AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA	60
20	GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC	120
	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	168
25	GTG GAC GGC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 20 25 30	216
30	CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	264
35	AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60	312
40	GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly 65 70 75 80	360
40	TAC AAG GOG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu 85 90 95	408
15	GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu 100 105 110	456
50	GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys 115 120 125	504
55	GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp 130 135 140	552
. 0	CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu ly 145 150 155 160	600
50	TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro	648

	GA(Asj	C CAC	G TG	G GCC P Ala 180	y vel	TAC Tyr	C CGC	G GCC G Ala	CTC a Let 189	2 Th	C GGG	G GAG Y As	GAG P G1	TCC u Se 19	r A	C AAC Sp Asn	69
5	Let	r ccc	GGG G1: 19:	y van	AAG Lys	GGC Gly	ATC / Ile	GGG Gly 200	GIL	AAG 1 Lyi	ACC Th	G GCC r Al	AGG A Arc	3 Ly	CT B. Le	T CTG	74
10	GAC Glu	G GAC 1 Glu 210	ırı	G GGG P Gly	AGC Ser	CTG Leu	GAA Glu 215	I WIS	CTC	CTC Lev	AAG Ly:	8 AAC 8 ABI 220	n Lei	GAC As	CG P Ar	G CTG g Leu	79:
15	AAG Lys 225	Pro	GCC Ala	ATC A Ile	CGG Arg	GAG Glu 230	LYE	ATC Ile	CTG Leu	GCC Ala	CAC His 23!	s Met	GAC Asp	GAT As _j	CTC	G AAG u Lys 240	840
20	CTC Leu	Ser	Tr	GAC Asp	Leu 245	Ala	AAG Lys	GTG Val	CGC Arg	ACC Thr 250	yei	CTG Leu	CCC Pro	CTG Let	GA0 1 G1 25	GTG u Val 5	888
	Asp	TTC Phe	GCC Ala	AAA Lys 260	Arg	CGG Arg	GAG Glu	CCC Pro	GAC Asp 265	Arg	GAG Glu	AGG Arg	CTT Leu	AGG Arg 270	Al	ATT a Ile	936
25	CTG Leu	GAG Glu	AGG Arg 275	Leu	GAG Glu	TTT Phe	GGC Gly	AGC Ser 280	Pro	CTC Leu	CAC His	GAG Glu	TTC Phe 285	GGC Gly	CTI Le	CTG u Leu	984
30	GAA Glu	AGC Ser 290	Pro	AAG Lys	GCC Ala	CTG Leu	GAG Glu 295	GAG Glu	GCC Ala	CCC Pro	TGG Trp	CCC Pro 300	Pro	CCG Pro	GAA Glu	GGG 1 Gly	1032
35	GCC Ala 305	rne	GTG Val	GGC Gly	TTT Phe	GTG Val 310	CTT Leu	TCC Ser	CGC Arg	AAG Lys	GAG Glu 315	Pro	ATG Met	TGG Trp	GCC Ala	GAT Asp 320	1080
40	CTT Leu	CTG Leu	GCC Ala	CTG Leu	GCC Ala 325	GCC Ala	GCC Ala	AGG Arg	GGG Gly	GGC Gly 330	CGG Arg	GTC Val	CAC His	CGG Arg	GCC Ala 335	Pro	1128
	GAG Glu	CCT Pro	TAT Tyr	AAA Lys 340	GCC Ala	CTC Leu	AGG Arg	GAC Asp	CTG Leu 345	AAG Lys	GAG Glu	GCG Ala	CGG Arg	GGG Gly 350	CTT Leu	CTC Leu	1176
45	GCC Ala	AAA Lys	GAC Asp 355	CTG Leu	AGC Ser	GTT Val	CTG Leu	GCC Ala 360	CTG Leu	AGG Arg	GAA Glu	GGC Gly	CTT Leu 365	GGC Gly	CTC Leu	CCG Pro	1224
50	CCC Pro	GGC Gly 370	GAC Asp	GAC Asp	CCC Pro	ATG Met	CTC Leu 375	CTC Leu	GCC Ala	TAC Tyr	CTC Leu	CTG Leu 380	GAC Asp	CCT Pro	TCC Ser	AAC Asn	1272
55	ACC Thr 385	ACC Thr	CCC Pro	GAG Glu	Gly	GTG Val 390	GCC Ala	CGG Arg	CGC Arg	TAC (GGC Gly 395	GGG Gly	GAG : Glu	rgg Trp	ACG Thr	GAG Glu 400	1320
60	GAG Glu	GCG Ala	GGG Gly	GAG lu	CGG Arg 405	GCC Ala	GCC Ala	CTT Leu	TCC Ser	GAG : Glu 410	AGG Arg	CTC Leu	TTC (Phe	SCC . Ala	AAC Asn 415	Leu	1368
	TGG Trp	GGG Gly	AGG Arg	CTT Leu 420	GAG (GGG Gly	GAG Glu	Glu	AGG (Arg	CTC (Leu	CTT Leu	TGG Trp	CTT : Leu	TAC	CGG Arg	GAG Glu	1416

								GTC Val 440	Leu					Ala		GGG Gly		1464
5													Leu			GCC Ala		1512
10								GCC Ala		Val		Arg				CAC His 480	. 1.	1560
15						Ser		Asp.			Glu					Asp		1608
20					Pro			GGC Gly		Thr					Lys			1656
20								GAG Glu 520										1704
25			Lys					CGG Arg										1752
30								CTC Leu										1800
35								GCC Ala										1848
40								ATC Ile										1896
70								GCC Ala 600										1944
45								CTC Leu										1992
50								TTC Phe										2040
55								GGC Gly										2088
60	CTG Leu	ATG Met	CGC Arg	CGG Arg 660	GCG Ala	GCC Ala	AAG Lys	ACC Thr	ATC Ile 665	AAC Asn	TTC Phe	GGG ly	GTC Val	CTC Leu 670	TAC Tyr	GGC Gly		2136
30								CAG Gln 680									:	2184

	GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg 690 695 700	2232
5	GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val 705 710 715 720	2280
10	GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG Glu Thr Leu Phe Gly Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 725 730 735	2328
15	GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro 740 745 750	2376
20	GTC CAG GGC ACC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu 765	2424
	TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 770 775 780	2472
25	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 790 795 800	2520
30	CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro 805 810 815	2568
35	CTG GAG GTG GAG GTG GGG ATA GGG GAC GAC TGG CTC TCC GCC AAG GAG Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 830	2616
	TGATACCACC	2626
40	(2) INFORMATION FOR SEQ ID No::4:	
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 832 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: protein	
50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::4:	
	Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	
55	Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 20 25 30	
	Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	
60	Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60	

	Val 65	Phe	Asp	Ala	Lys	Ala 70		Ser	Phe	Arg	His 75	Glu	Ala	Tyr	Gly	G13
5	Tyr	Lys	Ala	Gly	Arg 85	Ala	Pro	Thr	Pro	Glu 90	Asp	Phe	Pro	Arg	Gln 95	
	Ala	Leu	Ile	Lys 100	Glu	Leu	Val	Asp	Leu 105	Leu	Gly	Leu	Ala	Arg 110	Leu	Glu
10	Val	Pro	Gly 115	Tyr	Glu	Ala	Asp	Asp 120	Val	Leu	Ala	Ser	Leu 125	Ala	Lys	Lye
15	Ala	Glu 130	Lys	Glu	Gly	Tyr	Glu 135	Val	Arg	Ile	Leu	Thr 140	Ala	Asp	Lys	Asp
	Leu 145	Tyr	Gln	Leu	Leu	Ser 150	Asp	Arg	Ile	His	Val 155	Leu	His	Pro	Glu	Gly 160
20	Tyr	Leu	Ile	Thr	Pro 165	Ala	Trp	Leu	Trp	Glu 170	Lys	Tyr	Gly	Leu	Arg 175	Pro
	Asp	Gln	Trp	Ala 180	yab	Tyr	Arg	Ala	Leu 185	Thr	Gly	Asp	Glu	Ser 190	yab	Asn
25	Leu	Pro	Gly 195	Val	Lys	Gly	Ile	Gly 200	Glu	Lys	Thr	Ala	Arg 205	Lys	Leu	Leu
30	Glu 	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu
- ::	Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240
35	Leu	Ser	Trp	Asp	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	Val
	Asp	Phe	Ala	Lys 260	Arg	Arg	Glu	Pro	Asp 265	Arg	Glu	Arg	Leu	Arg 270	Ala	Ile
40	Leu	Glu	Arg 275	Leu	Glu	Phe	Gly	Ser 280	Pro	Leu	His	Glu	Phe 285	Gly	Leu	Leu
45	Glu	Ser 290	Pro	Lys	Ala	Leu	Glu 295	Glu	Ala	Pro	Trp	Pro 300	Pro	Pro	Glu	Gly
	Ala 305	Phe	Val	Gly	Phe	Val 310	Leu	Ser	Arg	Lys	Glu 315	Pro	Met	Trp	Ala	Asp 320
50	Leu	Leu	Ala	Leu	Ala 325	Ala	Ala	Arg	Gly	Gly 330	Arg	Val	His	Arg	Ala 335	Pro
	Glu	Pro	Tyr	Lys 340	Ala	Leu	Arg	Asp	Leu 345	Lys	Glu	Ala	Arg	Gly 350	Leu	Leu
55	Ala	Lys	Asp 355	Leu	Ser	Val	Leu	Ala 360	Leu	Arg	Glu	Gly	Leu 365	Gly	Leu	Pro
60	Pro	Gly 370	Asp	Asp	Pro	Met	Leu 375	Leu	Ala	Tyr	Leu	Leu 380	yab	Pro	Ser	Asn
	Thr 385	Thr	Pro	Glu	Gly	Val 390	Ala	Arg	Arg		Gly 395	Gly	Glu	Trp	Thr	Glu 400

					40	-				41	U				41	
5	Tr	p Gl	y Ar	420	u Gl	u G 1	y Gl	u Gl	u Ar 42	g Le 5	u Le	u Tr	p Le	u Ty 43	r Ar	g Glu
	V	al G	lu A: 439	g Pı	co La	eu S	er A	la V. 44	al L	eu A	la H	is M	et G 44	lu A 5	la T	hr Gly
10		450	•				45:	•				46	0			l Ala
15	•••	•				4/(47:)	1			His 480
					405	٠			: 73	, 49 0					49	
20	Glu	ı Leu	Gly	Leu 500	Pro	Ala	Ile	Gly	Lys 505	Thr	Glu	Lys	Thi	Gly 510		Arg
	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg	Glu	Ala 525	a His	Pro	Ile
25	Val	Glu 530	Lys	Ile	Leu	Gln	Tyr 535	Arg	Glu	Leu	Thr	Lys 540	Leu	Lys	Ser	Thr
30	Tyr 545	Ile	Asp	Pro	Leu	Pro 550	Asp	Leu	Ile	His	Pro 555	Arg	Thr	Gly	Arg	Leu 560
	His	Thr	Arg	Phe	Asn 565	Gln	Thr	Ala	Thr	Ala 570	Thr	Gly	Arg	Leu	Ser 575	Ser
35	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Leu 590		Gln
	Arg	Ile	A rg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala
40	Leu	Asp 610	Tyr	Ser	Gln	Ile	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
45	Asp 625	Glu	Asn	Leu	Ile	Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640
	Glu	Thr	Ala	Ser	Trp 645	Met	Phe	Gly	Val	Pro 650	Arg	Glu	Ala	Val	Asp 655	Pro
50	Leu	Met	Arg	Arg 660	Ala	Ala	Lys	Thr	Ile 665	Asn	Phe	Gly	Val	Leu 670	Tyr	Gly
·	Het	Ser	Ala 675	His	Arg	Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 685	Tyr	Glu	Glu
55	Ala	Gln 690	Ala	Phe	Ile	Glu	Arg 695	Tyr	Phe	Gln	Ser	Phe 700	Pro	Lys	Val	Arg
60	Ala 705	Trp	Ile	Glu	Lys	Thr 710	Leu	Glu	Glu	Gly	Arg 715	Arg	Arg	Gly	туг	Val 720
	Glu	Thr	Leu	Phe	Gly 725	Arg	Arg	Arg	Tyr	Val 730	Pro	Asp	Leu	Glu	Ala 735	

	Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro 740 745 750	
5	Val Gln Gly Thr Ala Ala Asp Leu Het Lys Leu Ala Het Val Lys Leu 755 760 765	
	Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 770 775 780	
10	Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 790 795 800	-
15	Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro 805 810 815	
	Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 830	
20	(2) INFORMATION FOR SEQ ID NO::5:	
25	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 2626 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
30	(iii) HYPOTHETICAL: NO	
	(iv) ANTI-SENSE: NO	
35	(vi) ORIGINAL SOURCE: (A) ORGANISM: Thermus aquaticus	
40	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(89, "g") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at postion 89 of the native Tag polymerase nucleotide sequence of C to G."</pre>	DNZ
45	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(934, "a") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native 1</pre>	ľac
50	DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."	
	(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(962, "c")	
55	(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native T DNA polymerase nucleotide sequence of T to C. This results in an amino acid change of Leu to Pro."	`aq
. 60	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(2535, "a") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>	

nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."

5	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(193, "t") (D) OTHER INFORMATION: /note= "This mutation changes the nucleotide at position 193 of the native Taq DNA</pre>	
10	polymerase from C to T, resulting in an amino acid chof Arg to Cys."	ıang
15	 (ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(504, "a") (D) OTHER INFORMATION: /note= "This mutation changes the nucleotide at position 504 of the native Taq DNA polymerase from G to A, which is conservative in natu 	re.
20	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1212619	
25	(ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 1212616	
30	(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 12619 (D) OTHER INFORMATION: /note= "pTarf3"	
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::5: AAGCTCAGAT CTACCTGCCT GAGGGGGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA	60
	GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGTGGTG GAAGGGTAAC	120
40	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	168
45.	GTG GAC GGC CAC CAC GCC TAC TGC ACC TTC CAC GCC CTG AAG GGC Val Asp Gly His His Leu Ala Tyr Cys Thr Phe His Ala Leu Lys Gly 20 25 30	216
50	CTC ACC ACC AGC CGG GGG GAC CCG GTG CAG GCG GTC TAC GGC TTC GCC Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	264
55	ANG AGC CTC CTC ANG GCC CTC ANG GAG GAC GGG GAC GCG GTG ATC GTG Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60	312
	GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly 65 70 75 80	360
50	TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GA	408

	GCC Ala	Leu	ATC Ile	Lys 100	Glu	CTG Leu	GTG Val	GAC ABI	CTC Lev 105	Let	GGG 2 Gly	CTG Le	GCG u Al	CGC a Ar 11	g Le	GAG u Glu	45
5	GTC Val	Pro	GGC Gly 115	Tyr	GAG Glu	GCG Ala	GAC Asp	GAC Asp 120	• Val	CTG Leu	GCC Ala	AGC Se:	CTC Level 12:	u Al	a Ly	S AAA s Lys	50
10	GCG Ala	GAA Glu 130	Lys	GAG Glu	GGC Gly	TAC	GAG Glu 135	Val	CGC Arg	ATC Ile	CTC Lev	ACC Thi	Ala	GAC As	AA p Ly	GAC B Asp	55:
15	CTT Leu 145	Tyr	CAG Gln	CTC Leu	CTT Leu	TCC Ser 150	Asp	CGC Arg	ATC Ile	CAC	GTC Val 155	Let	CAC His	CCC Pro	GAG Gl	GGG U Gly 160	600
20						Ala			Trp		Lys	Tyr		/ Le		CCC T	648
20											GGG Gly				As	AAC Asn	696
25				Val					Glu		ACG Thr			Lys		CTG Leu	744
30			Trp								AAG Lys		Leu			CTG Leu	792
35											CAC His 235						840
40											GAC Asp					Val	888
40											GAG Glu						936
45											CAC His						984
50											TGG Trp						- 1032
55											GAG Glu 315						1080
~ 0											CGG Arg						1128
60					Ala						GAG Glu						1176

	GC: Ala	C AA	A GA B As 35	b re	G AGO	C GTT	CTC	G GCC u Ala 360	a re	AGO Aro	GAA G Gl	GGC U G1	CT y Le 36	u Gl	CT y Le	C CCG	1224
5	Pro	GGG G1: 370	Y AS	C GAC	Pro	ATC Met	CTC Let 379	ı re	GCC Ala	TAC Tyl	CTC r Le	CTG Le	u As	C CCI	TC OSe	C AAC	1272
10	ACC Thi 385	Tn	Pro	GAG Glu	GGG Gly	GTG Val 390	. Als	CGG Arg	CGC Arg	TAC Tyr	GGC Gly 395	/ Gly	GAG Gl	TGG Tr	ACC P Th	G GAG r Glu 400	1320
15	GIU	Ala	r GT	/ Glu	405	Ala	Ale	Leu	ser	410	Arg	, Leu	Phe	≥ Ala	41		1368
20	Trp	, GIĀ	Arg	420	Glu	GIY	GIU	GIU	425	Leu	Leu	Trp	Leu	430	Ar	GAG g Glu	1416
	GTG Val	GAG Glu	AGG Arg 435	Pro	CTT Leu	TCC Ser	GCT Ala	GTC Val 440	Leu	GCC Ala	CAC His	ATG Met	GAG Glu 445	Ala	ACG Thi	GGG Gly	1464
25	GTG Val	CGC Arg 450	reu	GAC Asp	GTG Val	GCC Ala	TAT Tyr 455	Leu	AGG Arg	GCC Ala	TTG Leu	TCC Ser 460	Leu	GAG Glu	GTG Val	GCC L Ala	1512
30	GAG Glu 465	Glu	ATC Ile	GCC Ala	CGC Arg	CTC Leu 470	GAG Glu	GCC Ala	GAG Glu	GTC Val	TTC Phe 475	CGC Arg	CTG Leu	GCC Ala	GGC	CAC His 480	1560
35	CCC Pro	TTC Phe	AAC Asn	CTC Leu	AAC Asn 485	TCC Ser	CGG Arg	GAC Asp	CAG Gln	CTG Leu 490	GAA Glu	AGG Arg	GTC Val	CTC Leu	TTT Phe 495	Asp	1608
40	GAG Glu	CTA Leu	GGG Gly	CTT Leu 500	CCC Pro	GCC Ala	ATC Ile	GGC Gly	AAG Lys 505	ACG Thr	GAG Glu	AAG Lys	ACC Thr	GGC Gly 510	AAG Lys	CGC Arg	1656
	TCC Ser	ACC Thr	AGC Ser 515	GCC Ala	GCC Ala	GTC Val	CTG Leu	GAG Glu 520	GCC Ala	CTC Leu	CGC Arg	GAG Glu	GCC Ala 525	CAC His	CCC Pro	ATC Ile	1704
45	GTG Val	GAG Glu 530	AAG Lys	ATC Ile	CTG Leu	CAG Gln	TAC Tyr 535	CGG Arg	GAG Glu	CTC . Leu	ACC . Thr	AAG Lys 540	CTG Leu	AAG Lys	AGC Ser	ACC Thr	1752
50	TAC Tyr 545	ATT Ile	GAC Asp	CCC Pro	TTG Leu	CCG Pro 550	GAC Asp	CTC . Leu	ATC (His	CCC : Pro 555	AGG A	ACG Thr	GGC Gly	CGC Arg	CTC Leu 560	1800
55	CAC His	ACC Thr	CGC Arg	TTC Phe	AAC Asn 565	CAG Gln	ACG Thr	GCC Ala	Thr	GCC A Ala 570	ACG (Thr	GC (AGG Arg	CTA /	AGT Ser 575	AGC Ser	1848
50	TCC Ser	GAT Asp	CCC Pro	AAC Asn 580	CTC Leu	CAG . Gln	AAC . Asn	Ile	CCC (Pro 585	GTC (Val	CGC A	ACC (Thr	Pro	CTT (Leu 590	GGG Gly	CAG Gln	1896
	AGG Arg	ATC Ile	CGC Arg 595	CGG Arg	GCC Ala	TTC . Phe	Ile	GCC (Ala 600	GAG (Glu (GAG (Glu (GG 1	Trp	CTA : Leu 605	MG (Leu	GTG Val	GCC Ala	1944

	Lev	GAC - Asp 610	Tyr	AGC Ser	CAG Glr	ATA 11e	GAG Glu 615	ı. Lei	AGG Arg	GTG Val	CTG Le	GCC Ala 620	a Hi	C CTC s Le	C;TCC u Se	c GGC er Gly	1992
, 5	GAC Asp 625	Glu	'Asn	CTG Leu	ATC	CGG Arg 630	Val	Phe	CAG Gln	GAG Glu	GGG G1 ₃ 635	Arg	GAC As	ATC P Il	CAC e Hi	B Thr 640	2040
10	GAG Glu	ACC	GCC	AGC Ser	TGG Trp 645	Met	TTC Phe	GGC Gly	GTC Val	CCC Pro 650	Arg	GAG Glu	GCC Ala	GTG A Va	GAC 1 As 65	CCC p Pro 5	2088
15	CTG Leu	ATG Met	CGC	CGG Arg 660	Ala	GCC Ala	AAG Lys	ACC Thr	ATC Ile 665	Asn	TTC Phe	GGG Gly	GTC Val	CTC Let 670	Ty:	GGC Gly	2136
20	ATG Met	TCG Ser	GCC Ala 675	CAC His	CGC Arg	CTC Leu	TCC Ser	CAG Gln 680	Glu	CTA Leu	GCC Ala	ATC Ile	CCT Pro 685	Tyr	GAG Glu	GAG 1 Glu	2184
20	GCC Ala	CAG Gln 690	GCC Ala	TTC Phe	ATT Ile	GAG Glu	CGC Arg 695	TAC Tyr	TTT Phe	CAG Gln	AGC Ser	TTC Phe 700	Pro	AAG Lys	GTG Val	CGG L Arg	223 2
25	GCC Ala 705	Trp	ATT Ile	GAG Glu	AAG Lys	ACC Thr 710	CTG Leu	GAG Glu	GAG Glu	GGC Gly	AGG Arg 715		CGG Arg	GGG Gly	TAC	GTG Val 720	2280
30	GAG Glu	ACC Thr	CTC Leu	TTC Phe	GGC Gly 725	CGC Arg	CGC Arg	CGC Arg	TAC.	GTG Val 730	CCA Pro	GAC Asp	CTA Leu	GAG Glu	GCC Ala 735	Arg	2328
35	GT Val	Lys	S AGO Ser	Val 740	CGG Arg	GAG Glu	GCG Ala	GCC Ala	GAG Glu 745	CGC Arg	ATG Met	GCC Ala	TTC Phe	AAC Asn 750	ATG Met	CCC Pro	2376
40	GTC Val	CAG Gln	GGC Gly 755	ACC Thr	GCC Ala	GCC Ala	GAC Asp	CTC Leu 760	ATG Met	AAG Lys	CTG Leu	GCT Ala	ATG Met 765	GTG Val	AAG Lys	CTC Leu	2424
40	TTC Phe	CCC Pro 770	AGG Arg	CTG Leu	GAG Glu	GAA Glu	ATG Met 775	GGG Gly	GCC Ala	AGG Arg	ATG Met	CTC Leu 780	Leu	CAG Gln	GTC Val	CAC His	2472
45	GAC Asp 785	GAG Glu	CTG Leu	GTC Val	CTC Leu	GAG Glu 790	GCC Ala	CCA Pro	AAA Lys	GAG Glu	AGG Arg 795	GCG Ala	GAG Glu	GCC Ala	GTG Val	GCC Ala 800	2520
50												CCC Pro		Ala			2568
55												CTC '					2616
	TGAT	LYCCI	cc														2626

60 (2) INFORMATION FOR SEQ ID NO::6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 amino acids

(B) TYPE: amino acid (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5			(11)	MOI	PECOT	E T	PE:	pro	ein							
•		(xi)	SEQU	JENCE	DES	SCRIE	PTIO	i: s	EQ II	ON C	:6:			•	
10	Met 1	Arg	Gly	/ Met	Leu 5	Pro	Lev	Phe	Glu	Pro	Lys	G1;	y Ar	y Val	l Le	
	Val	Asp	Gly	His 20	His	Leu	ı Ala	Туг	Cys	Thi	Phe	His	a Ala	Let 30		3 G1
15	Leu	Thr	Thr 35	Ser	Arg	Gly	Glu	Pro 40	Val	Glr	Ala	Va]	Tyr 45	Gly	Phe	≥ Ala
	Lys	Ser 50	Leu	Leu	Lys	Ala	Leu - 55	Lys	Glu	Asp	Gly	Asr 60	Ala	Val	Ile	• Val
20	Val 65	Phe	Asp	Ala	Lys	Ala 70	Pro	Ser	Phe	Arg	His .75	Glu		Tyr	Gly	Gly BC
25	Tyr	Lys	Ala	Gly	Arg 85	Ala	Pro	Thr	Pro	Glu 90	Asp	Phe	Pro	Arg	Gln 95	
	Ala	Leu	Ile	Lys 100	Glu	Leu	Val	Asp	Leu 105	Leu	Gly	Leu	Ala	Arg 110		Glu
30	Val	Pro	Gly 115	Tyr	Glu	Ala	Asp	Asp 120	Val	Leu	Ala	Ser	Leu 125	Ala	Lys	Lys
	Ala	Glu 130	Lys	Glu	Gly	Tyr	Glu 135	Val	Arg	Ile	Leu	Thr 140	Ala	Asp	Lys	Asp
35	Leu 145	Tyr	Gln	Leu	Leu	Ser 150	Asp	Arg	Ile	His	Val 155	Leu	His	Pro	Glu	Gly 160
40	Tyr	Leu	Ile	Thr	Pro 165	Ala	Trp	Leu	Trp	Glu 170	Lys	Tyr	Gly	Leu	Arg 175	Pro
	Asp	Gln	Trp	Ala 180	Asp	Tyr	Arg	Ala	Leu 185	Thr	Gly	Asp	Glu	Ser 190	Asp	Asn
45	Leu	Pro	Gly 195	Val	Lys	Gly	Ilę	Gly 200	Glu	Lys	Thr	Ala	Arg 205	Lys	Leu	Leu
	Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu
50	Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240
55	Leu	Ser	Trp	Asp	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	Val
	Asp	Phe	Ala	Lys 260	Arg	Arg	Glu	Pro	Asp 265	Arg	Glu	Arg		Arg 270	Ala	Ile
60	Leu	Glu	Arg 275	, Leu	Glu	Phe	Gly	Ser 280	Pro	Leu	His		Phe 285	Gly	Leu	Leu
	Glu	Ser 290	Pro	Lys	Ala	Leu	Glu 295	G1u	Ala	Pro	Trp	Pro	Pro	Pro	Glu	Gly

	Ala 305	Phe	Val	Gly	Phe	Val 310	Leu	Ser	Arg	Lys	Glu 315		Met	Trp	Ala	As:
5	Leu	Leu	Ala	Leu	Ala 325	Ala	Ala	Arg	Gly	Gly 330		Val	His	Arg	Ala 335	
	Glu	Pro	Tyr	Lys 340	Ala	Leu	Arg	Asp	Leu 345	Lys	Glu	Ala	Arg	Gly 350	Leu	Let
10	Ala	Lys	Asp 355	Leu	Ser	Val	Leu	Ala 360	Leu	Arg	Glu	Gly	Leu 365	Gly	Leu	Pro
15	Pro	Gly 370	Asp	Asp	Pro	Met	Leu 375	Leu	Ala	Tyr	Leu	Leu 380	Авр	Pro	Ser	Aer
.,	Thr 385	Thr	Pro	Glu	Gly	Val 390	Ala	Arg	Arg	Tyr	Gly 395	Gly	Glu	Trp	Thr	G10 400
20	Glu	Ala	Gly		Arg 405		Ala		Ser	Glu 410	Arg	Leu	Phe	Ala	Asn 415	Leu
	Trp	Gly	Arg	Leu 420	G]u	Gly	Glu	Glu	Arg 425	Leu	Leu	Trp	Leu	Tyr 430	Arg	Glu
25	Val	Glu	Arg 435	Pro	Leu	Ser	Ala	Val 440	Leu	Ala	His	Het	Glu 445	Ala	Thr	Gly
30	Val	Arg 450	Leu	Asp	Val	Ala	Tyr 455	Leu	Arg	Ala	Leu	Ser 460	Leu	Glu	Val	Ala
	Glu 465	Glu	Ile	Ala	Arg	Leu 470	Glu	Ala	Glu	Val	Phe 475	Arg	Leu	Ala	Gly	His 480
35					485					Leu 490					495	
	Glu	Leu	Gly	Leu 500	Pro	Ala	Ile	Gly	Lys 505	Thr	Glu	Lys	Thr	Gly 510	Lys	Arg
40	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg	Glu	Ala 52 5	His	Pro	lle
		530	•				535	_		Leu		540		_		
45	Tyr 545	Ile	Àsp	Pro	Leu	Pro 550	Asp	Leu	Ile	His	Pro 555	Arg	Thr	Gly	Arg	Leu 560
	His	Thr	Arg	Phe	Asn 569		Thr	Ala .	Thr	Ala 570		Gly	Arg	Leu	Ser 575	
50	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Let 590	Gly	Gln
55	Arg	Ile	Arg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala
	Leu	Asp 610	Tyr	Ser	Gln	Ile	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
60	Asp 625	lu	Asn	Leu				Phe		Glu	Gly 635	Arg	Asp	Ile	His	Thr 640

(ix) FEATURE:

	Glu	Thr	Ala	Ser	Trp 645	Met	Phe	Gly	Val	Pro 650	Arg	,Glu	ı İ Ä Li	a Val	1 Ası 659	Pro	
. 5				300					003					670	٠.	Gly	
			. 675				٠.	000					685			Glu	
10		030	•			Glu	033					700				•	
15	,03					Thr 710					/15					720	
					125	Arg				/30					735	-	
20				740		Glu			745					750			
			/55			Ala		760					765				
25		,,,					//5					780					
30	703					Glu 790					795					800	
	Arg				6U3					810					815		
35	Leu	Glu	Val 3	Glu 820	Val	Gly :	Ile	Gly	Glu 825	Asp	Trp :	Leu		Ala 830	Lys	Glu	
40	(2)	INFO	RMAT	ION	FOR	SEQ :	ID N	0::7	:								
40		(i)	(A (B) LE	NGTH PE: 1	ARACT 262 nucle EDNES	26 ba	ase ;	pair:	6							٠
45	1	(ii)	(D)	TO	POLO	GY: 1 PE: [ine	ar -)							
						L: NO		-	,	•							
50	((iv)	ANT	-SEI	NSE:	NO											
	((VI)				JRCE: SM: T		nus a	iquat	icus							
55		•	(B)	NAN	Æ/KE CATIO	Y: m N: r INFO	epla	ce (8	19, * : /n	'g") iote=	- - T	his	mut	atio		sults	in
60	nucle	otio	ie		alte	rati mera	on a	t po	stic	n 89	of	the	nati	V6 T	'a~ h		

	 :	
5	 (A) NAME/KEY: mutation (B) LOCATION: replace(934, "a") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native T DNA polymerase nucleotide sequence of T.to A. This results in an amino acid change of Phe to Ile." 	aç
10	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(962, "c") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native To DNA polymerase nucleotide sequence 67 T to C. This results in an amino acid change of Leu to Pro."</pre>	aq
15		
20	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(2535, "a") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>	ıq
25	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(341, "a") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>	p
30	DNA polymerase nucleotide sequence of G to A. This mutation results in an amino acid change of Arg to His.	
35	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1212619	
	(ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 1212616	
40	(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 12619 (D) OTHER INFORMATION: /note= "pTarf5"	
45	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::7:	
	AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA 6	0
50	GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC 12	0
	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG Het Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	8
55	GTG GAC GGC CAC CTG GCC TAC CGC ACC TTC CAC CC CTG AAG GGC 21	6
	Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 20 25 30	•
50	CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45	4

	AAG Lys	AGG Ser 50	Let	Leu	AAG Lye	GCC Ala	CTC Let 59	LY	G GAG B Gl	G GA	c GG(p Gl	G GAC y As	p Al	G GT a Va	G A1	c c	TG /al	312
5	GTC Val 65	Pne	GAC Asp	GCC Ala	AAG Lys	GCC Ala 70	CCC Pro	TCC Sea	TTC r Ph	CAC Hi	CAC B Hi	B Gl	GCC u Al	C TA a Ty	c çç	G G	GG Hy 80	360
10	TAC	Lys	GCG Ala	GGC	CGG Arg 85	Ala	CCC Pro	ACG Thr	Pro	GAG Gli	ı Ası	TTT P Phe	CCC	CGG O Ar	g G	A Ci ln L	rc eu	408
15	GCC Ala	CTC	ATC Ile	AAG Lys 100	Glu	CTG Leu	GTG Val	Asp	CTC Let 109	Leu	GGG Gly	CTG Leu	GCG Ala	CGC Ar 11	g Le	C G#	iG lu	456
20	ANT	Pro	115	ıyr	GIU	AIA	GAC Asp	120	Val	Leu	Ala	Ser	125	Al.	a Ly	s L	ys	504
	GCG Ala	GAA Glu 130	rys	GAG Glu	GGC Gly	TAC Tyr	GAG Glu 135	GTC Val	CGC Arg	ATC	CTC Leu	ACC Thr 140	Ala	GAC Asj	AAI D Ly	A GA s A:	c sp	552
25	CTT Leu 145	TAC Tyr	CAG Gln	CTC Leu	CTT Leu	TCC Ser 150	GAC Asp	CGC Arg	ATC Ile	CAC His	GTC Val 155	Leu	CAC His	CCC	GAC Gl	GG u G1	y	600
30	TAC Tyr	CTC Leu	ATC Ile	ACC Thr	CCG Pro 165	GCC Ala	TGG Trp	CTT Leu	TGG Trp	GAA Glu 170	Lys	TAC Tyr	GGC Gly	CTG Lev	AGG Ar	g Pr	C C	648
35	GAC Asp	CAG Gln	TGG Trp	GCC Ala 180	GAC Asp	TAC Tyr	CGG Arg	GCC Ala	CTG Leu 185	ACC Thr	GGG Gly	GAC Asp	GAG Glu	TCC Ser 190	As	AA As	e n	696
40	CTT Leu	CCC Pro	GGG Gly 195	GTC Val	AAG Lys	GGC Gly	ATC Ile	GGG Gly 200	GAG Glu	AAG Lys	ACG Thr	GCG Ala	AGG Arg 205	AAG Lys	CTT	CIO Le	; u	744
	GAG Glu	GAG Glu 210	TGG Trp	GGG Gly	AGC Ser	CTG Leu	GAA Glu 215	GCC Ala	CTC Leu	CTC Leu	AAG Lys	AAC Asn 220	CTG Leu	GAC Asp	CGG Arg	CTC Le	; u	792
15	AAG Lys 225	CCC Pro	GCC Ala	ATC Ile	Arg	GAG Glu 230	AAG Lys	ATC Ile	CTG Leu	GCC Ala	CAC His 235	ATG Met	Asp GAC	GAT Asp	CTG Leu	AAG Ly 24	В	840
50	CTC Leu	TCC Ser	TGG Trp	GAC Asp	CTG Leu 245	GCC Ala	AAG Lys	GTG Val	CGC Arg	ACC Thr 250	GAC Asp	CTG (Leu	CCC Pro	CTG Leu	GAG Glu 255	Va.	ì	888
55	GAC Asp	TTC Phe	ATa	AAA Lys 260	AGG Arg	CGG Arg	GAG (Glu	Pro	GAC Asp 265	CGG Arg	GAG . Glu	AGG (Arg	CTT . Leu	AGG Arg 270	GCC Ala	ATT	•	936
	CTG ·	GAG Glu	AGG Arg 275	CTT Leu	GAG ' Glu	TTT (Phe	Gly	AGC (Ser 280	CCC Pro	CTC Leu	CAC (Glu	TTC (Phe 285	GGC Gly	CTT Leu	CTG Let	1	984
.•	GAA . Glu	AGC Ser 290	CCC Pro	AAG Lys	GCC (Ala	Leu	GAG (Glu (295	GAG (GCC (Ala	CCC '	Trp	CCC (Pro 300	CCG (Pro	CCG Pro	GAA Glu	GGG Gly	,	1032

	A1 a 305	Phe	Val	GGC	Phe	Val 310	Let	ı Se	r Arg	Lys	GAG G1: 31:	u Pro	O Me	t Tr	F Al	C GAT A Asp 320	1080
5						Ala					/ Arq					CCC a Pro 5	1128
10	GAG Glu	Pro	TAT Tyr	Lys 340	Ala	CTC Leu	AGG	GAC Asp	CTG Leu 345	Lys	GAG Glu	GCG Ala	CGG Arg	GGG G13 350	Le	CTC u Leu	1176
15	GCC Ala	Lys	GAC Asp 355	Leu	AGC Ser	GTT Val	CTG	GCC Ala 360	Leu	AGG Arg	GAA Glu	GGC Gly	CTT Leu 365	ı Gly	CTC / Le	CCG u Pro	1224
20	CCC Pro	GGC Gly 370	Asp	GAC Asp	CCC Pro	ATG Met	CTC Leu 375	Leu	GCC Ala	TAC Tyr	CTC	CTG Leu 380	Asp	CCT Pro	TCC Sea	AAC Asn	1272
		Thr										Gly				GAG Glu 400	1320
25			GGG Gly													Leu	1368
30	TGG Trp	GGG Gly	AGG Arg	CTT Leu 42	Glu	GGG Gly	GAG Glu	GAG Glu	AGG Arg 42	Leu	CTT Leu	TGG Trp	CTT Leu	TAC Tyr 43	Arg	GAG Glu	1416
35	GTG Val	GAG Glu	AGG Arg 435	CCC Pro	CTT Leu	TCC Ser	GCT Ala	GTC Val 440	CTG Leu	GCC Ala	CAC His	ATG Met	GAG Glu 445	GCC Ala	ACG Thr	GGG	1464
40			CTG Leu													GCC Ala	1512
70	GAG Glu 465	GAG Glu	ATC Ile	GCC Ala	CGC Arg	CTC Leu 470	GAG Glu	GCC Ala	GAG Glu	GTC Val	TTC Phe 475	CGC Arg	CTG Leu	GCC Ala	GGC Gly	CAC His 480	1560
45	CCC Pro	TTC Phe	AAC Asn	CTC Leu	AAC Asn 485	TCC Ser	CGG Arg	GAC Asp	CAG Gln	CTG Leu 490	GAA Glu	AGG Arg	GTC Val	CTC Leu	TTT Phe 495	GAC Asp	1608
50	GAG Glu		GGG Gly														1656
55	TCC Ser	ACC Thr	AGC Ser 515	GCC Ala	GCC Ala	GTC Val	CTG Leu	GAG Glu 520	GCC Ala	CTC Leu	CGC Arg	GAG Glu	GCC Ala 525	CAC His	CCC Pro	ATC Ile	1704
40			AAG Lys			Gln											1752
60	TAC Tyr 545	Ile	GAC Asp	CCC Pro	TTG Leu	CCG Pro 550	Asp	CTC Leu	ATC Ile	CAC His	Pro 555	Arg	ACG (Thr	GGC (Gly	CGC Arg	CTC Leu 560	1800

	CAC His	ACC	CGC Arg	TTC	AAC ABD 565	Glr	ACG Thr	GCC	ACG Thi	GCC Ala 570	Th	GGC G1	AGO Y Ar	CT! g. Le	A AG: u Se 57	F AGC r Ser 5	1848
5	TCC	GAT Asp	Pro	AAC Asn 580	Leu	CAG Glm	AAC	ATC	CCC Pro 589	Val	CGC Arg	ACC Thi	CCG Pre	CTI Le 59	u Gl	G CAG y Gln	1896
10	AGG Arg	ATC Ile	CGC Arg 595	Arg	GCC Ala	TTC Phe	ATC	GCC Ala 600	Glu	GAG Glu	GGG Gly	TGG Tr	CTA Let 609	Le	GTC U Va	GCC l Ala	1944
15	CTG Leu	GAC Asp 610	TAT	AGC Ser	CAG Gln	ATA Ile	GAG Glu 615	Leu	AGG Arg	GTG Val	CTG Leu	GCC Ala 620	His	CTC Let	TCC Se	GGC Gly	1992
20	GAC Asp 625	GAG Glu	AAC Asn	CTG Leu	ATC Ile	CGG Arg 630	Val	TTC Phe	CAG Gln	GAG Glu	GGG Gly 635	' Arg	GAC Asp	ATC Ile	CAC His	ACG Thr 640	2040
	GAG Glu	ACC Thr	GCC Ala	AGC Ser	TGG Trp 645	ATG Met	TTC Phe	GGC Gly	GTC Val	CCC Pro 650	CGG Arg	GAG Glu	GCC Ala	GTG Val	GAC Asp 655	Pro	2088
25	CTG Leu	ATG Met	CGC Arg	CGG Arg 660	GCG Ala	GCC Ala	AAG Lys	ACC Thr	ATC Ile 665	AAC Asn	TTC Phe	GGG Gly	GTC Val	CTC Leu 670	Tyr	GCC	2136
30	ATG Met	TCG Ser	GCC Ala 67	His	CGC Arg	CTC Leu	TCC Ser	CAG Gln 68	Glu	CTA Leu	GCC Ala	ATC Ile	CCT Pro 68	Tyr	GAG Glu	GAG Glu	2184
35	GCC Ala	CAG Gln 690	GCC Ala	TTC Phe	ATT Ile	GAG Glu	CGC Arg 695	TAC Tyr	TTT Phe	CAG Gln	AGC Ser	TTC Phe 700	CCC Pro	AAG Lys	GTG Val	CGG Arg	2232
10	GCC Ala 705	TGG Trp	ATT Ile	GAG Glu	AAG Lys	ACC Thr 710	CTG Leu	GAG Glu	GAG Glu	GGC Gly	AGG Arg 715	AGG Arg	CGG Arg	GGG Gly	TAC Tyr	GTG Val 720	2280
•	GAG Glu	ACC Thr	CTC Leu	TTC Phe	GGC Gly 725	CGC Arg	CGC Arg	CGC Arg	TAC Tyr	GTG Val 730	CCA Pro	Asp Asp	CTA Leu	GAG Glu	GCC Ala 735	CGG Arg	2328
15	GTG Val	AAG Lys	AGC Ser	GTG Val 740	CGG Arg	GAG Glu	GCG Ala	GCC Ala	GAG Glu 745	CGC . Arg	ATG Met	GCC Ala	TTC Phe	AAC Asn 750	ATG Met	CCC Pro	2376
50	GTC Val	CAG Gln	GGC Gly 755	ACC Thr	GCC Ala	GCC Ala	GAC Asp	CTC Leu 760	ATG Met	AAG Lys	CTG Leu	GCT Ala	ATG Met 765	GTG Val	AAG Lys	CTC Leu	2424
55	Phe	CCC Pro 770	AGG Arg	CTG Leu	GAG Glu	GAA Glu	ATG Met 775	GGG Gly	GCC Ala	AGG :	ATG Met	CTC Leu 780	CTT Leu	CAG Gln	GTC Val	CAC His	2472
60	GAC Asp 785	GAG Glu	CTG Leu	GTC Val	CTC Leu	GAG Glu 790	GCC Ala	CCA Pro	AAA Lys	GAG : Glu	AGG Arg 795	GCG Ala	GAG Glu	GCC Ala	GTG Val	GCC Ala 800	2520
	CGG Arg	CTG Leu	GCC Ala	AAG Lys	GAA Glu 805	Val	Met	Glu	Gly	GTG : Val 810	Tyr	CCC Pro	CTG Leu	GCC Ala	GTG Val	Pro	2568

	CTG	GAG Glu	GTG Val	GAG Glu 820	Val	GGG Gly	ATA Ile	GGG Gly	GAG Glu 825	ı Ası	TGG Tr	CTC Le	TCC Se	GCC R Al	a Ly	GAG B Glu	2616
5	TGA	TACC	ACC												•		2626
	(2)	INF	ORMA	TION	FOR	SEQ	ΙĎ	NO::	8:								
10			(1)	(A) LE	CHA NGTH PE: POLO	: 83 amin	2 aπ	ino id	: acid	8						
15		(ii)	MOLE	CULE	TYP	E: p	rote	in								
		(xi)	SEQU	ENCE	DES	CRIP	TION	: SE	Q ID	NO:	:8:	-				
20	Met 1		Gly	Met	Leu 5	Pro	Leu	Phe	Glu	Pro 10		Gly	Arg	Val	Leu 15	Leu	
	Val	Asp	Gly	His 20		Leu	Ala	Tyr	Arg 25	Thr	Phe	His	Ala	Leu 30		Gly	
25	Leu	Thr	Thr 35		Arg	Gly	Glu	Pro 40		Gln	Ala	Val	Tyr 45	Gly	Phe	Ala	
30	Lys	Ser 50	Leu	Leu	Lys	Ala	Leu 55	Lys	Glu	Asp	Gly	Asp 60	Ala	Val	Ile	Val	
50	Val 65	Phe	Asp	Ala	Lys	Ala 70	Pro	Ser	Phe	His	His 75	Glu	Ala	Tyr	Gly	Gly 80	
35	Tyr	Lys	Ala	Gly	Arg 85	Ala	Pro	Thr	Pro	Glu 90	Asp	Phe	Pro	Arg	Gln 95	Leu	
	Ala	Leu	Ile	Lys 100	Glu	Leu	Val	Asp	Leu 105	Leu	Gly	Leu	Ala	Arg 110	Leu	Glu	
40	Val	Pro	Gly 115	Tyr	Glu	Ala	Asp	Asp 120	Val	Leu	Ala	Ser	Leu 125	Ala	Lys	Lys	
45	Ala	Glu 130	Lys	Glu	Gly	Tyr	Glu 135	Val	Arg	Ile	Leu	Thr 140	Ala	Asp	Lys	Asp	
75	Leu 145	Tyr	Gln	Leu	Leu	Ser 150	Asp	Arg	Ile	His	Val 155	Leu	His	Pro	Glu	Gly 160	
50	Tyr	Leu	Ile	Thr	Pro 165	Ala	Trp	Leu	Trp	Glu 170	Lys	Tyr	Gly	Leu	Arg 175	Pro	
	Asp	Gln	Trp	Ala 180	Asp	Tyr	Arg	Ala	Leu 185	Thr	Gly	Asp	Glu	Ser 190	Asp	Asn	
55	Leu	Pro	Gly 195	Val	Lys	Gly	Ile	Gly 200	Glu	Lys	Thr	Ala	Arg 205	Lys	Leu	Leu	•
60	Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu	
00	Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240	

	Leu	Ser	Trp	Asp	Leu 245	Ala	Lye	Val	Arg	Thr 250	yet	Leu	Pro	Le	G1: 25:	u Val
5	Авр	Phe	Ala	Lys 260	Arg	Arg	Glu	Pro	Asp 265	Arg	Glu	Arg	Leu	270		lle
	Leu	Glu	Arg 275	Leu	Glu	Phe	Gly	Ser 280	Pro	Leu	His	Glu	Phe 285	Gly	/ Let	1 Leu
10	Glu	Ser 290	Pro	Lys	Ala	Leu	Glu 295	Glu	Ala	Pro	Trp	Pro 300	Pro	Pro	Glu	Gly
15	Ala 305	Phe	Val	Gly	Phe	Val 310	Leu	Ser	Arg	Lys	Glu 315	. Pro	Het	Trp	Ala	320
	Leu	Leu	Ala	Leu	Ala 325	Ala	Ala	Arg	Gly	Gly 330	Arg	Val	His	Arg	Ala 335	
20	Glu	Pro	Tyr	Lys 340	Ala	Leu	Arg	Asp	Leu 345	Lys	Glu	Ala	Arg	Gly 350		Leu
	Ala	Lys	Asp 355	Leu	Ser	Val	Leu	Ala 360	Leu	Arg	Glu	Gly	Leu 365	Gly	Leu	Pro
25	Pro	Gly 370	Asp	Asp	Pro	Met	Leu 375	Leu	Ala	Tyr	Leu	Leu 380	Asp	Pro	Ser	Asn
30	Thr 385	Thr	Pro	Glu	Gly	Val 390	Ala	Arg	Arg	Tyr	Gly 395	Gly	Glu	Trp	Thr	Glu 400
	Glu	Ala	Gly	Glu	Arg 405	Ala	Ala	Leu	Ser	Glu 410	Arg	Leu	Phe	Ala	Asn 415	Leu
35	Trp	Gly	Arg	Leu 420	Glu	Gly	Glu	Glu	Arg 425	Leu	Leu	Trp	Leu	Tyr 430	Arg	Glu
	Val	Glu	Arg 435	Pro	Leu	Ser	Ala	Val 440	Leu	Ala	His	Met	Glu 445	Ala	Thr	Gly
40	Val	Arg 450	Leu	Asp	Val	Ala	Tyr 455	Leu	Arg	Ala	Leu	Ser 460	Leu	Glu	Val	Ala
45	Glu 465	Glu	Ile	Ala	Arg	Leu 470	Glu	Ala	Glu	Val	Phe 475	Arg	Leu	Ala	Gly	His 480
	Pro	Phe	Asn	Leu`	Asn 485	Ser	Arg	Asp	Gln	Leu 490	Glu	Arg	Val	Leu	Phe 495	Asp
50	Glu	Leu	Gly	Leu 500	Pro	Ala	Ile	Gly	Lys 505	Thr	Glu	Lys	Thr	Gly 510	Lys	Arg _.
	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg		Ala 525	His	Pro	Ile
55	Val	Glu 530	Lys	Ile	Leu	Gln	Tyr 535	Arg	Glu	Leu		Lys 540	Leu	Lys	Ser	Thr
50	Tyr 545	Ile	Asp	Pro	Leu	Pro 550	Asp	Leu	Ile	His	Pro 555	Arg	Thr	Gly		Leu 560
	His	Thr	Arg		Asn 565	Ğln	Thr	Ala		Ala 570	Thr	Gly	Arg	Leu	Ser 575	Ser

	Ser	Asp	Pro	Asn 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	Leu 590	Gly	Gln
5	Arg	Ile	Arg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala
	Leu	Asp 610	Tyr	Ser	Gln	Ile	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
10	Asp 625	Glu	Asn	Leu	Ile	Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640
15	Glu	Thr	Ala	Ser	Trp 645	Met	Phe	Gly	Val	Pro 650	Arg	Glu	Ala	Val	Asp 655	Pro
13	Leu	Met	Arg	Arg 660	Ala	Ala	Lys	Thr	11e 665	Asn	Phe	Gly	Val	Leu 670	Tyr	Gly
20	Met	Ser	Ala 675	His	Arg	Leu	Ser	Gln 680	Glu	Ļeu	Ala	Ile	Pro 685	Tyr	Glu	Glu
	Ala	Gln 690	Ala	Phe	Ile	Glu	Arg 695	Tyr	Phe	Gln	Ser	Phe 700	Pro	Lys	Val	Arg
25	Ala 705	Trp	lle	Glu	Lys	Thr 710	Leu	Glu	Glu	Gly	Arg 715	Arg	Arg	Gly	Tyr	Val 720
30	Glu	Thr	Leu	Phe	Gly 725	Arg	Arg	Arg	Tyr	Val 730	Pro	Asp	Leu	Glu	Ala 735	Arg
	Va]	Lys	s Ser	Val	Arg	Glu	Ala	Ala	Glu 745	Arç	Met	Ala	Phe	Asn 750	Met	Pro
35	Val	Gln	Gly 755	Thr	Ala	Ala	Asp	Leu 760	Met	Lys	Leu	Ala	Met 765	Val	Lys	Leu
	Phe	Pro 770	Arg	Leu	Glu	Glu	Met 775	Gly	Ala	Arg		Leu 780	Leu	Gln	Val	His
40	Asp 785	Glu	Leu	Val		Glu 790	Ala	Pro	Lys	Glu	Arg 795	Ala	Glu	Ala		Ala 800
45	Arg	Leu	Ala	Lys	Glu 805	Val	Met	Glu	Gly	Val 810	Tyr	Pro	Leu	Ala	Val 815	Pro
40	Leu	Glu	Val	Glu 820	Val	Gly	Ile		Glu 825	Asp	Trp	Leu		Ala 830	Lys	Glu
50	(2)	INFO	RMAT	NOI	FOR	SEQ	ID N	0::9	:					_		
55		(1)	() (E) LE) TY) SI	E CH NGTH PE: RAND	: 26 nucl EDNE	26 b eic SS:	ase acid sing	pair	s						
		(333		·	POLO E TY				omic	.)						
60		-			TICA			, , ,	_,							
	•		FEA													

(A) NAME/KEY: mutation

	 (B) LOCATION: replace(89, "g") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at postion 89 of the native Tapolymerase nucleotide sequence of C the "golymerase 	C DNA
5	polymerase nucleotide sequence of C to G.	d DIV
	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(934, "a") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration."</pre>	
10	DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."	Taq
15	(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(962, "c") (D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native	
20	DNA polymerase nucleotide sequence of T to C. This results in an amino acid change of Leu to Pro."	rad
25	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(2535, "a") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>	Taq
30	<pre>(ix) FEATURE: (A) NAME/KEY: mutation (B) LOCATION: replace(337, "a") (D) OTHER INFORMATION: /note= "This mutation results in a</pre>	
35	nucleotide alteration at position 337 of the native DNA polymerase nucleotide sequence of T to C. This char results in an amino acid change of Phe to Leu."	Taq ınge
40	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1212619	
45	(ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 1212616 (ix) FEATURE: (A) NAME/KEY: mat_peptide (A) NAME/KEY: mat_peptide	
50	(A) NAME/REY: - (B) LOCATION: 12619 (D) OTHER INFORMATION: /note= "pTarf2"	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::9:	
55	AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA	60
		120
60	ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG Het Arg Gly Het Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15	168
	GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTC AAC GCC	

	Val	Asp	Cly	His 20		Leu	Ala	Туг	Arg 25	Thr	Phe	Hie	Ala	Lei 30	Ly:	s Gly	
5	CTC Leu	ACC Thr	ACC Thr 35	Ser	CGG Arg	GGG Gly	GAG Glu	CCG Pro 40	Val	CAG Gln	GCG Ala	GTC Val	TAC Tyr 45	GGC Gly	TTC Phe	GCC ≥ Ala	264
10			Leu					Lys								GTG Val	312
15	GTC Val 65	Phe	GAC Asp	GCC Ala	AAG Lys	GCC Ala 70	CCC Pro	TCC	CTC Leu	CGC Arg	CAC His 75	Glu	GCC Ala	TAC Tyr	GGG Gly	GGG Gly 80	360
.,	TAC Tyr	AAG Lys	GCG Ala	GGC Gly	CGG Arg 85	GCC	CCC	ACG Thr	CCG Pro	GAG Glu 90	GAC Asp	TTT Phe	CCC Pro	CGG Arg	CAA Gln 95	Leu	408
20					GAG Glu										Leu	GAG Glu	456
25					GAG Glu									Ala		AAG Lys	504
30					GGC 1 Gly											a Aap	552
35					CTT Leu												600
33					CCG Pro 165												648
40					GAC Asp												696
45					AAG Lys												744
50					AGC Ser												792
• •					CGG Arg												840
55					CTG Leu 245												888
60					AGG Arg												936

	CTG	GAC Glu	AGG Arg 275	Leu	GAG	TTT Phe	GGC Gly	AGC Ser 280	Pro	CTC Leu	CAC His	GAG Glu	TTC Phe 28	e Gl	CTI Le	CTG u Leu	984
5	GAA Glu	AGC Ser 290	Pro	AAG Lys	GCC Ala	CTG Leu	GAG Glu 295	Glu	GCC Ala	CCC Pro	TGG Trp	CCC Pro 300	Pro	CCG Pro	GAA Gl	GGG 1 Gly	1032
10	GCC Ala 305	Phe	GTG Val	GGC Gly	TTT Phe	GTG Val 310	Leu	TCC Ser	CGC Arg	AAG Lys	GAG Glu 315	Pro	ATG Met	TGG Trp	GCC Ala	GAT Asp 320	1080
15	CTT Leu	Leu	GCC Ala	CTG Leu	GCC Ala 325	GCC Ala	GCC Ala	AGG Arg	GGG Gly	GGC Gly 330	Arg	GTC Val	CAC	CGG Arg	GCC Ala 335	Pro	1128
20	GAG Glu	Pro	TAT	Lys 340	Ala	CTC Leu	AGG Arg	GAC Asp	CTG Leu 345	AAG Lys	GAG Glu	GCG Ala	CGG Arg	GGG Gly 350	CTT Leu	CTC Leu	1176
	GCC Ala	AAA Lys	GAC Asp 355	Leu	AGC Ser	GTT Val	CTG Leu	GCC Ala 360	CTG Leu	AGG Arg	GAA Glu	GGC Gly	CTT Leu 365	GGC Gly	CTC Leu	CCG Pro	1224
25	CCC Pro	GGC Gly 370	GAC Asp	GAC Asp	CCC Pro	ATG Met	CTC Leu 375	CTC Leu	GCC Ala	TAC Tyr	CTC Leu	CTG Leu 380	GAC Asp	CCT Pro	TCC Ser	AAC Asn	1272
30	ACC Thr 385	ACC Thr	CCC Pro	GAG Glu	GGG Gly	GTG Val 390	GCC Ala	CGG Arg	CGC Arg	TAC Tyr	GGC Gly 395	GGG Gly	GAG Glu	TGG Trp	ACG Thr	GAG Glu 400	1320
35	GAG Glu	GCG Ala	GGG Gly	GAG Glu	CGG Arg 405	GCC Ala	GCC Ala	CTT Leu	TCC Ser	GAG Glu 410	AGG Arg	CTC Leu	TTC Phe	GCC Ala	AAC Asn 415	CTG Leu	1368
40	TGG Trp	GGG Gly	AGG Arg	CTT Leu 420	GAG Glu	GGG Gly	GAG Glu	GAG Glu	AGG Arg 425	CTC Leu	CTT Leu	TGG Trp	CTT Leu	TAC Tyr 430	CGG Arg	GAG Glu	1416
	GTG Val	GAG Glu	AGG Arg 435	CCC Pro	CTT Leu	TCC Ser	GCT Ala	GTC Val 440	CTĞ Leu	GCC Ala	CAC His	ATG Met	GAG Glu 445	GCC 7	ACG Thr	GGG Gly	1464
45	GTG Val	CGC Arg 450	CTG Leu	yab GyC	GTG Val	GCC Ala	TAT Tyr 455	CTC Leu	AGG Arg	GCC Ala	TTG : Leu	TCC (Ser 460	CTG (Leu	GAG (Glu	STG (Val	GCC Ala	1512
50	GAG Glu 465	GAG Glu	ATC Ile	GCC Ala	C GC A rg	CTC Leu 470	GAG (Glu	GCC Ala	GAG (Glu	GTC Val	TTC (Phe 475	OGC (Arg	CTG (Leu	GCC (Ala	GC (CAC His 480	1560
55	CCC Pro	TTC Phe	AAC Asn	CTC Leu	AAC Asn 485	TCC Ser	CGG (Arg	GAC Asp	CAG (Gln	CTG (Leu 490	GAA I Glu	AGG (Arg	GTC (Val	Leu	TTT (Phe 495	GAC Asp	1608
••	GAG Glu	CTA Leu	GGG Gly	CTT Leu 500	CCC Pro	GCC . Ala	ATC (Gly	AAG A Lys 505	ACG (Thr	GAG 1 Glu	AAG 1 Lys	Thr	GC F Gly 510	AAG (Lys	SC Arg	1656
60	TCC Ser	ACC Thr	AGC Ser 515	GCC Ala	GCC (GTC (CTG C	Glu	CC C	TC C Leu	GC G Arg	Glu .	CC C Ala :	AC C	CC A	TC Ile	1704

	GTG Val	GAG Glu 530	Lys	ATC	CTG Leu	CAG Gln	TAC Tyr 535	Arc	GAG Glu	CTC Leu	ACC Thr	AAG Lys 540	: Lei	AAG Lyi	AGC S Se:	ACC r Thr	1752
5	TAC Tyr 545	Ile	GAC Asp	CCC Pro	TTG Leu	CCG Pro 550	Asp	CTC Leu	ATC Ile	CAC His	CCC Pro 555	Arç	ACG Thr	GGC Gly	cec / Arg	CTC G Leu 560	1800
10	CAC His	ACC Thr	CGC Arg	TTC Phe	AAC Asn 565	Gln	ACG Thr	GCC	ACG Thr	GCC Ala 570	Thr	GCC	AGG Arg	CTA Leu	AGT Ser 575	AGC Ser	1848
15										Val					Gly	CAG Gln	1896
20	Arg	Ile	Arg 595	Arg	Ala		Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605	Leu	Val	Ala	1944
	Leu	Asp 610	Tyr	Ser	Gln		Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly	1992
25	Asp 625	Glu	Asn	Leu	Ile	630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640	2040
30	Glu	Thr	Ala	Ser	Trp 645		Phe	Gly	Val	Pro 650	Arg	Glu	Ala	Val	Asp 655	Pro	2088
35	Leu	Met	Arg	Arg 660	Ala		Lys	Thr	11e 665	Asn	Phe	Gly	Val	Leu 670	Tyr	Gly	2136
40	Met	Ser	Ala 675	His	Arg	CTC Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 6 85	Tyr	Glu	Glu	2184
						GAG Glu											2232
45						ACC Thr 710											2280
50	Glu	Thr	Leu	Phe	Gly 725	CGC Arg	Arg	Arg	Tyr	Val 730	Pro	Asp	Leu	Glu	Ala 735	Arg	2328
55						GAG Glu											2376
60						GCC Ala											2424
•*						GAA 2 Glu					Met						2472

	GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG A8p Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val 785 790 795	GCC 2520 Ala 800
5	CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val 805 810	. Pro
10	CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys 820 825 830	GAG 2616 Glu
	TGATACCACC	2626
15	(2) INFORMATION FOR SEQ ID NO::10:	
20	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 832 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: protein	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::10:	
,	Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu 1 5 10 15	Leu
30	Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys 20 25 30	Gly
35	Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe 35 40 45	Ala
	Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile 50 55 60	Val
40	Val Phe Asp Ala Lys Ala Pro Ser Leu Arg His Glu Ala Tyr Gly 65 70 75	Gly 80
	Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln 85 90 95	Leu
45	Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu 100 105 110	Glu
50	Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys 115 120 125	Lys
	Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys 130 135 140	Asp
55	Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu 145 150	Gly 160
	Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg 165 170 175	Pro
60	Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp 180 185 190	Asn

	Leu	Pro	Gly 195	Val	Lys	Gly	Ile	Gly 200	Glu	Lys	Thr	Ala	Arg 205	Lys	Leu	Le
5	Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg •	Le
	Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240
10	Leu	Ser	Trp	yab	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	
15	Asp	Phe	Ala	Lys 260	Arg	Arg	Glu	Pro	Asp 265	Arg	Glu	Arg	Leu	Arg 270	Ala	Ile
.,	Leu	Glu	Arg 275	Leu	Glu	Phe	Gly	Ser 280	Pro	Leu	His	Glu	Phe 285	Gly	Leu	Leu
20	Glu	Ser 290	Pro	Lys	Ala	Leu	Glu 295	Glu	Ala	Pro	Trp	Pro 300	Pro	Pro	Glu	Gly
	Ala 305	Phe	Val	Gly	Phe	Val 310	Leu	Ser	Arg	Lys	Glu 315	Pro	Met	Trp	Ala	320
25					325					330	Arg				335	
30				340					345		Glu			350		
		_	355					360			g Glu		365			•
35		370					375				Leu	380				
•	385					390					Gly 395					400
40			_		405					410	Arg				415	
45				420			•		425		Leu			430		
			435					440			His		445			
50		450					455				Leu	460		-		
;	465				_	470					Phe 475					480
55					485					490	Glu				495	
60				500					505		Glu			510		
	Ser	Thr	Ser 515	Ala	Ala	Val	Leu	Glu 520	Ala	Leu	Arg	Glu	Ala 525	His	Pro	Ile

	Val	. Glu 530	Lys	Ile	Leu	Gln	Tyr 535	Arc	Glu	ı Lei	1 Thr	Lye 540	Leu	Ly:	s Se	Thr
5	Tyr 545	Ile	Asp	Pro	Leu	Pro 550	Asp	Leu	Ile	Hi:	555	Arg	Thr	Gly	Arq •	Leu 560
	His	Thr	Arg	Phe	Asn 565	Gln	Thr	Ala	Thr	570	Thr	Gly	Arg	Leu	Ser 575	
10	Ser	Asp	Pro	Aen 580	Leu	Gln	Asn	Ile	Pro 585	Val	Arg	Thr	Pro	590		Gln
15	Arg	Ile	Arg 595	Arg	Ala	Phe	Ile	Ala 600	Glu	Glu	Gly	Trp	Leu 605		Val	Ala
••	Leu	Asp 610	Tyr	Ser	Gln	Ile	Glu 615	Leu	Arg	Val	Leu	Ala 620	His	Leu	Ser	Gly
20	Asp 625	Glu	Asn	Leu	Ile	Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640
	Glu	Thr	Ala	Ser	Trp 645	Met	Phe	Gly	Val	Pro 65	Arg O	Glu	Ala	Val	Asp 65	
25	Leu	Met	Arg	Arg 660	Ala	Ala	Lys	Thr	Ile 665	Asn	Phe	Gly	Val	Leu 670	Tyr	Gly
30	Met	Ser	Ala 675	His	Arg	Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 685	Tyr	Glu	Glu
		Gln 690					695					700				
35	Ala 705	Trp	Ile	Glu	Lys	Thr 710	Leu	Glu	Glu	Gly	Arg 715	Arg	Arg	Gly	Tyr	Val 720
		Thr			725					730					735	
40		Lys		740					745					750		
45		Gln	755					760					765			
		Pro 770					775					780				
50	785	Glu				790					795					800
		Leu			805					810					815	
55	Leu	Glu	Val	Glu 820	Val	Gly	Ile	Gly	Glu 825	Asp	Trp	Leu		Ala 830	Lys	Glu

(2) INFORMATION FOR SEQ ID NO::11:

60

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid

		(C) STRANDEDNESS: single (D) TOPOLOGY: linear	
,	(ii)	MOLECULE TYPE: CDNA	
5	(iii)	HYPOTHETICAL: NO	
10	(ix)	FEATURE: (A) NAME/KEY: - (B) LOCATION: 118 (D) OTHER INFORMATION: /note= "PCR reverse primer used for PUC18"	
15	(xi)	SEQUENCE DESCRIPTION: SEQ ID NO::11:	
	CAGGAAAC	RG CTATGACC	1
20	(2) INFO	RMATION FOR SEQ ID NO::12:	
25	(1)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii)	MOLECULE TYPE: cDNA	
30	·(iii)	HYPOTHETICAL: NO	
35	(ix)	FEATURE: (A) NAME/KEY: - (B) LOCATION: 1115 (D) OTHER INFORMATION: /note= "PCR sequencing primer 628A used for pUC18"	
40	(xi)	SEQUENCE DESCRIPTION: SEQ ID NO::12:	
	CCCAAAGC	CA GGCCG	,15
45	(2) INFO	RMATION FOR SEQ ID NO::13:	
50	(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double	
30	/ / / /	(D) TOPOLOGY: linear MOLECULE TYPE: cDNA	
55	(111)	HYPOTHETICAL: NO	
60	(ix)	FEATURE: (A) NAME/KEY: - (B) LOCATION: 115 (D) OTHER INFORMATION: /note= "Sequencing primer 1155A"	
		CROHENCE DESCRIPTION. SPO ID NO13.	

	CAGGTCCCTG AGGGC	15
	(2) INFORMATION FOR SEQ ID NO::14:	
5	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 46 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single	
10	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
	(iii) HYPOTHETICAL: NO	
15	(ix) FEATURE: (A) NAME/KEY: -	
20	(B) LOCATION: 146 (D) OTHER INFORMATION: /note= "pUC18 - pLSM5 5' junction"	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::14:	
25	AATTTCACAC AGGAAACAGC TATGACCATG ATTACGAATT CTARAA	46
23	(2) INFORMATION FOR SEQ ID NO::15:	
30	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 63 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
35	(ii) MOLECULE TYPE: cDNA	
	(iii) HYPOTHETICAL: NO	
40	<pre>(ix) FEATURE: (A) NAME/KEY: - (B) LOCATION: 163 (D) OTHER INFORMATION: /note= "pUC18 - pLSM5 3' sequence</pre>	
45		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO::15:	
	CAAGGAGTGA GATTCTCTAG AGTCGACCTG CAGGCATGCA AGCTTGGCAC TGGCCGTCGT	60
50	TIT	63

10

15

20

What is claimed is:

- A modified Taq DNA polymerase gene essentially comprising the native Taq DNA polymerase gene having an altered nucleotide at positions 89, 934, 962, and 2535.
- 2. A modified *Taq* DNA polymerase gene according to claim 1 wherein the altered nucleotide at position 89 is G, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A.
- 3. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 1.
 - 4. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 2.
 - A modified Taq DNA polymerase essentially comprising the native Taq
 DNA polymerase with an altered amino acid at positions 272 and 281.
 - 6. A modified *Taq* DNA polymerase according to claim 5 wherein the altered amino acid at position 272 is Ile and the altered amino acid at position 281 is Pro.
 - A modified Taq DNA polymerase gene essentially comprising the native
 Taq DNA polymerase gene having an altered nucleotide at positions 193 and 504.
 - 8. A modified *Taq* polymerase gene according to claim 7 wherein the altered nucleotide at position 193 is T and the altered nucleotide at position 504 is A.

- 9. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 193, 504, 934, 962, and 2535.
- 10. A modified *Taq* polymerase gene according to claim 9 wherein the altered nucleotide at position 89 is G, the altered amino acid at position 193 is T, the altered amino acid at position 504 is A, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A.
- 11. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 7.
 - 12. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 8.
 - Host cells transfected with the modified Taq DNA polymerase gene of claim 9.
- 15 14. Host cells transfected with the modified Taq DNA polymerase gene of claim 10.
 - 15. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at position 25.
 - 16. A modified Taq DNA polymerase according to claim 15 wherein the altered amino acid at position 25 is Cys.
 - 17. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at positions 25, 272, and 281

PCT/US94/13554

5

10

15

- 18. A modified *Taq* DNA polymerase according to claim 17 wherein the altered amino acid at position 25 is Cys, the altered amino acid at position 272 is Ile, and the altered amino acid at position 281 is Pro.
- 19. A modified *Taq* DNA polymerase gene essentially comprising the native

 Taq DNA polymerase gene having an altered nucleotide at position 341.
 - 20. A modified *Taq* polymerase gene according to claim 19 wherein the altered nucleotide at position 193 is T and the altered nucleotide at position 504 is A.
- 21. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 341, 934, 962, and 2535.
 - 22. A modified *Taq* polymerase gene according to claim 21 wherein the altered nucleotide at position 89 is G, the altered amino acid at position 341 is A, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A
 - 23. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 19.
 - 24. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 20.
 - 25. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 21.
 - 26. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 22.

5

10

- 27. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at position 74.
- 28. A modified Taq DNA polymerase according to claim 27 wherein the altered amino acid at position 74 is His.
- 29. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at positions 74, 272, and 281
- 30. A modified *Taq* DNA polymerase according to claim 29 wherein the altered amino acid at position 74 is His, the altered amino acid at position 272 is Ile, and the altered amino acid at position 281 is Pro.
- 31. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.
 - 32. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.
- 33. Polymerase chain reaction, wherein the improvement comprises use ofthe modified *Taq* DNA polymerase of claim 15.
 - 34. Polymerase chain reaction, wherein the improvement comprises use of the modified *Tag* DNA polymerase of claim 16.
 - 35. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.
 - 36. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18.
 - 37. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.

PCT/US94/13554

5

10

15

- 38. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 38.
- 39. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.
- 40. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.
- 41. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.
- 42. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.
- 43. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.
- 44. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.
- 45. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.
- 46. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18.
- 47. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.
- 48. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 28.

5

10

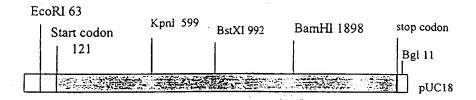
15

- 49. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.
- 50. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.
- 51. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.
- 52. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.
- 53. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.
 - 54. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.
 - 55. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.
 - 56. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18
 - 57. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.
 - 58. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 28.
 - 59. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.

60. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.

1/8

FIG. 1



Restriction map of gene for Taq DNA polymerase

FIG. 2
Scheme for Zone Mutagenesis

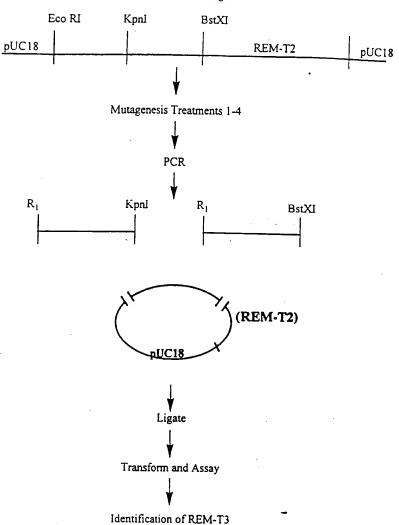
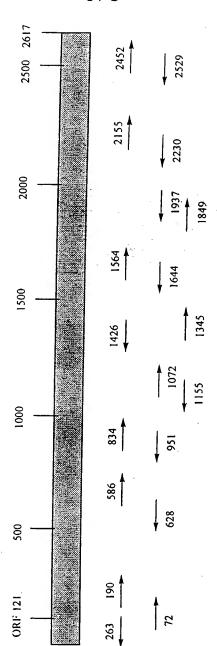
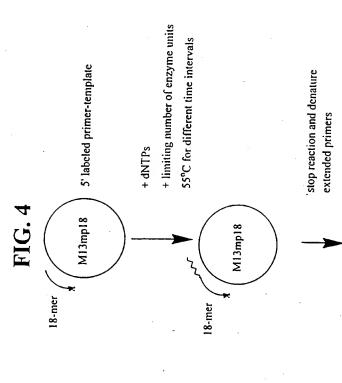


FIG. 3

Sequencing Primers for pLSM5





Polyacrylamide gel analysis of length of extended primers

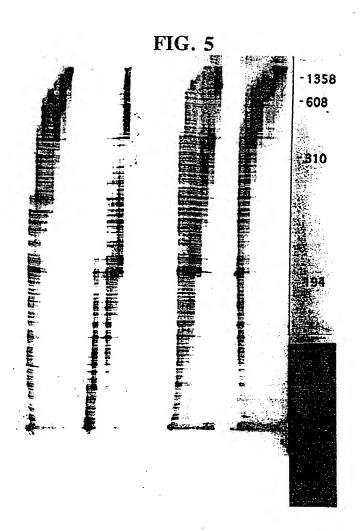


FIG. 6

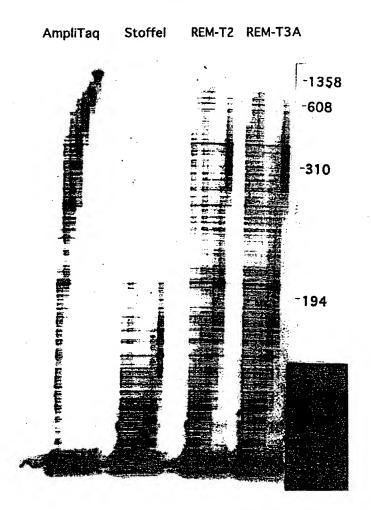


FIG. 7

PCR Analysis of Processivity

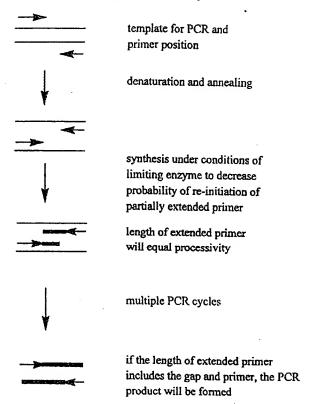


FIG. 8

